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HERBERT MCKENZIE DENSLOW

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Herbert McKenzie Denslow was born at Lynn, Massachusetts, August 20, 1852. As a boy he became interested in botany through association with his uncle, William Wallace Denslow (1826–1868). Together they collected plants, often in the wilds of upper Manhattan; both were among the original members of the Torrey Botanical Club, in 1867. The uncle died shortly after this time, but the nephew has maintained his botanical interests throughout the 75 years of the Club's existence.

Dr. Denslow graduated from Yale in 1873. He was given the degree of A.M. by Kenyon College in 1896, and that of D.D. by General Theological Seminary in 1907. In 1878 he became a deacon, in 1879 a priest in the Protestant Episcopal Church. From 1878 to 1902 he discharged the duties of a clergyman at various places in Connecticut, Vermont, western New York, Ohio, and Indiana. During these 24 years he was unable to take active part in the meetings of the Torrey Club; but he maintained the connection as corresponding member. In 1902 he returned to New York as Professor of Pastoral Theology in General Theological Seminary, in which capacity he served until his retirement as Professor Emeritus in 1923. For a part of this period he was sub-dean, and at times acting dean.

Dr. Denslow's botanical work has been particularly concerned with our native orchids. A considerable list of publications bears witness to his attainments in this field, and in his search for these plants he has visited nearly every town in the state of Connecticut. His studies have been intensified during his late years, since retirement from professional duties.

To all who enjoy the privilege of his acquaintance, he is outstanding as a profound yet tolerant scholar, and as a kindly and cordial gentleman. The Club honors itself in congratulating its oldest living member on his ninety years.

J.H.B.

The portrait of Dr. Denslow is published with the assistance of the Lucien M. Underwood Memorial Fund.

[The BULLETIN for June 69: 405–478 was issued June 2, 1942]

BULLETIN OF THE TORREY BOTANICAL CLUB
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NUCLEAR BEHAVIOR IN THE MUCORALES—I. THE MUCOR PATTERN

VICTOR M. CUTTER, JR.

For the past 60 years the question of nuclear behavior in the Mucorales has been a source of controversy. The exact stages in the life history at which nuclear fusion and the subsequent nuclear reduction take place have never been satisfactorily demonstrated. It is obvious that before further studies in the sexuality and genetics of this group can be carried out, these critical stages must be studied from a comparative basis and their position definitely established. A survey of the 50-odd publications dealing with this question indicates that the present confusion arises from four principal sources. The extremely minute nature of the mucoraceous nucleus, the use of unsatisfactory cytological techniques, the great natural variation in the material used in these investigations, and the failure of the majority of workers to follow the nuclear behavior through all the stages in the life history have all contributed to this controversy. This and a subsequent paper present the results of a correlated study of the nuclear condition in 15 species of this group at all stages in the life cycle.

MATERIALS AND METHODS

The species used in this study are listed below. An attempt has been made so far as possible to select species which have been studied previously in order that the results of the present investigation might be reasonably compared with those of earlier investigations, and also in the hope of correlating some of the divergent opinions on controversial points. The nomenclatural system followed is that of Naumov (21). Herbarium material of all the species studied has been deposited in the Herbarium of Plant Pathology at Cornell University and in the Farlow Herbarium of Cryptogamic Botany at Harvard University under the numbers indicated in the accompanying table.

Species investigated

No.	Species	No.	Species
25	*Mucor genevensis* Lendner	87	*Zygorhynchus vuillemenii* Namysl.
82	*Mucor hiemalis* Wehmer	90	*Zygorhynchus vuillemenii* var. *agamus* Namysl.
13	*Parasitella simplex* Bainier	7	*Absidia spinosa* Lendner
63	*Zygorhynchus dangeardi* Moreau	81	*Blakeslea trispora* Thaxter
68	*Zygorhynchus moelleri* Vuill.		

In order to secure as much uniformity as possible all material was cultured upon a single substrate, carrot decoction agar. Mature zygospores were

germinated by sterilizing them in 12 per cent calcium hypochlorite solution and isolating individual spores on 2 per cent non-nutrient agar, or moist filter paper in petri dishes after they had reached the proper age. A future publication concerning the details of this process and the results of a number of experiments upon the factors influencing germination of the zygospores of the Mucorales is anticipated.

Young mycelia, sporangia, and the early stages in zygospore formation were prepared for cytological examination by the method recommended by Schwarze (26) in his study of the water molds. The same technique was used in preparing the germ tubes of zygospores for sectioning. Older zygospores, chlamydospores, and hyphal bodies were lifted from the substrate attached to portions of the mycelium and immediately immersed in fixative. In such cases an aspirator was used to remove all air from the material, and to insure thorough penetration of the fixative. In this connection a detergent "Tergitol" Penetrant 7, supplied by the Carbide and Carbon Chemicals Corporation of New York was used in conjunction with all fixatives. The addition of three drops of this compound to every 50 cc. of the fixative was found to lower the surface tension of the solution to the point where almost instantaneous wetting of the material occurred. In addition, the penetration of the fixative into larger thick-walled structures was greatly enhanced.

As might be expected the most nearly natural and lifelike fixation images were produced by the various chromic-osmic-acetic mixtures, of which Flemming's weak solution and Chamberlain's solution as cited by Johansen (13) proved the most useful. Randolph's modification of Navashin's fluid cited under the abbreviation Craf (13) has proved excellent in studying the older zygote stages where it is difficult, because of excess reserve substances, to bleach out the osmic acid after fixation. Formalin-acetic-alcohol is very useful for the study of nuclear structure in young hyphae and zygospore stages. Gilson's fluid (13) and saturated mercuric chloride solution have been extensively used in conjunction with the Feulgen reaction. With this procedure they prove quite satisfactory but are practically worthless when followed by other stains.

For a study of the chondriome and of the cytoplasmic inclusions two solutions developed by Zirkle (31) have given the best results. Zirkle-Erliki fluid removes all chromatin and preserves mitochondria well, while Zirkle's reduced chromic fluid preserves vacuoles and mitochondria well and at the same time does not completely obliterate the chromatin present, so that a combined picture of the acid and basic images may be secured. Regaud's fluid as cited by Lee (16) occasionally gave satisfactory results but it was troublesome to use with the older stages and was not so generally employed as the two preceding formulae.

Dehydration of all materials, before they were embedded in paraffin, was carried out in the n-butyl alcohol series given by Zirkle (31). This method

proved much more satisfactory than did any of the xylene or chloroform series and tended to reduce shrinkage to a minimum. With the older zygospore stages it was necessary, because of the brittle nature of the exospore, to subject the material to a softening process before sectioning. The best results were obtained with material soaked in 3 per cent sodium hydroxide immediately after fixation. All materials were embedded in 56° paraffin, and sections cut from 3 to 15 microns thick.

It became obvious early in the course of this study that little would be gained by repeating the staining methods usual to this type of investigation, since the unsatisfactory results obtained by many workers indicated that Flemming's triple stain and Heidenhain's haematoxylin were not sufficiently precise to reveal nuclear detail during the stages when various structures were in the dormant condition. Hence a definite attempt was made to adapt more modern and precise staining techniques to these fungi. Several staining methods not previously employed in the study of the Mucorales have been used. The first of these and perhaps the most important was the Feulgen reaction. Great care must be used in the interpretation of structures showing a reaction to this test, for, as Knaysi (15) points out, the significance of this reaction is as yet not fully understood. The staining solution was prepared in accordance with Coleman's (7) modification of de Tomasi's method. The time of hydrolysis varies with the fixative employed, and in this connection Hillary's (12) schedule has been followed with very satisfactory results.

The second staining method used was based upon a technique described by Naylor (22) but different dyes and a different buffer series was used. The dyes selected were 0.5 per cent aqueous erythrosin and saturated aqueous methylene blue as suggested by Savile (25). His staining schedule was followed but the buffer selected was the acid potassium-phthalate series of Clark and Lub (6). This buffer was employed in place of the disodium-phosphate citric acid series recommended by Savile, because of the difficulties encountered with fungi growing in the latter solutions.

Hruby's modification of the Cahal-Brozek basic fuchsin-indigocarmin method (13) has proved to be a very rapid and practical stain for mycelium and young zygote stages in conjunction with any fixative. Samples of all the material stained by the three preceding methods were also stained in Heidenhain's iron alum–haematoxylin and in Flemming's triple stain for purposes of comparison. In all cases the results obtained by the newer techniques were equally satisfactory, and with respect to the stages in the life cycle where abundant reserve substances were present in the fungus structures these methods gave more precise results. For a study of the elements of the chondriome, staining with Heidenhain's iron alum–haematoxylin, and the mitochondrial method of Bensley-Cowdry cited by Lee (16), have proved generally satisfactory.

The illustrations in this paper have been made diagrammatic to the extent that no detail has been rendered in the cell walls, since this has no bearing on the present problem. However, important dimensions such as the relative thickness of the several zygospores wall layers have been portrayed as accurately as possible. The exospore has been shown in black, and the endospore has been left blank to secure a contrast between these two walls. Amorphous structures, such as oil vacuoles, have been left blank to distinguish them from the reticulately stippled cytoplasm. All drawings were made with a projection mirror or camera lucida adjusted to table top level and with Leitz apochromatic objectives and compensating eyepieces. Inasmuch as other workers on the same problem have figured many of the stages encountered in this study, no attempt has been made to reillustrate those stages which have previously been adequately portrayed.

Abbreviations. The following abbreviations of stains and fixatives are used in the legends accompanying the figures, to indicate the type of preparation from which the drawings and photographs were made.

Fixatives

Flemming's weak solution	Flemming's
Chamberlain's solution	Chamberlain's
Gilson's fluid	Gilson's
Saturated mercuric chloride	S.M.C.
Formalin-acetic-alcohol	F.A.A.
Randolph's modification of Navashin's fluid	Craf
Zirkle-Erliki fluid	Zirkle's
Zirkle's reduced chronic fluid	Z.R.C.

Stains

Feulgen reaction for nucleoprotein	Feulgen
Methylene blue and Erythrosin used with buffer solutions	Buffer
Cahal-Brozek basic fuchsin indigocarmin	Cahal-Brozek
Bensly-Cowdry acid fuchsin light green for mitochondria	Bensly-Cowdry
Flemming's triple stain	Triple
Heidenhain's iron-alum haematoxylin	Haematoxylin

Certain matters pertaining to the terminology of the various nuclear and cytoplasmic structures require clarification before proceeding to a detailed discussion of the species investigated. Baird (2) has presented a very complete review of the investigations on nuclear structure and consequently such a summary will be omitted here. As interpreted in the following study, a typical vegetative nucleus in the Mucorales consists of one or more deeply staining central bodies surrounded by a clear region which may or may not be bounded by an obvious membrane. Around the periphery of this clear region and within the membrane, if one is present, a limited number of chromatic threads are arranged indiscriminately. These threads rarely form a definite reticulum, and are only visible after very precise fixation. At the

present time it is impossible to state whether they represent the complete nuclear reticulum or are merely the basichromatic portions of a more extensive reticulum which has, as yet, not been demonstrated. These threads are Feulgen positive and have been found in all the metabolic nuclei examined. A nuclear membrane is apparently associated with all metabolic nuclei, although the small size of the nuclei and the fact that the membrane frequently collapses under the action of fixatives frequently make it difficult to detect.

The central body is the most prominent constituent of the nuclear unit. It has commonly been referred to as a nucleole or nucleolus, but by some authors (2) it has been interpreted as the entire nucleus. Whether this body can be considered homologous with the nucleolus of higher organisms is open to question. It is highly refractive and stains brilliant red with Flemming's triple stain in contrast to the bluish or purple reticulum; on this basis it has been considered a typical nucleolus. On the other hand in material subjected to the Feulgen reaction the central body is Feulgen positive, as is also the nuclear reticulum. During division it separates into two approximately equal portions and passes to the poles of the spindle in company with the chromatic elements of the reticulum. These facts would mitigate the nucleolus theory and perhaps suggest that the mucoraceous nucleus shows affinities with the karyosome nuclei of certain lower organisms (27). It is conceivable, however, that the reaction to the Feulgen test results from the collopse of a Feulgen positive portion of the reticulum around the central body; thus masking its true reaction. Until further light is thrown upon this matter it seems advisable to refrain from designating this structure the nucleolus, since this would imply that it is homologous with the nucleolus of higher organisms.

In the species considered below two types of nuclear division occur. In the rapidly growing portions of the thallus mitotic divisions take place. During division a very faint spindle is developed with the highly chromatic mass of the central body and presumably also of the nuclear reticulum lying in the equatorial region. The nuclear membrane becomes very faint at this stage but does not completely disappear: hence the achromatic figure may be considered intranuclear. The chromatic mass then separates into two approximately equal portions which move towards the poles of the spindle. As these masses reach the poles, a clear region forms around each of them, the spindle disappears and shortly thereafter a nuclear membrane is again apparent around each daughter nucleus. At no stage in this process has it been possible to distinguish definite chromosomes, although the chromatic masses during their migration to the poles are not entirely homogeneous. Occasionally during the early division stages refractive granules can be seen at or near the poles and outside the nuclear membrane. These structures probably represent centrioles, but their behavior before the onset of nuclear division and

after the reorganization of the daughter nuclei could not be determined. They are by no means universally present in these fungi as Moreau's account (18) might lead one to believe. The periodicity of mitotic division was very marked in all the forms examined and frequently it was possible to investigate large amounts of material without encountering any division stages.

In the older vegetative mycelium and the maturing and dormant zygospores a type of nuclear division which is perhaps best interpreted as amitosis occurs. Here the central body of the nucleus merely elongates, the nuclear membrane disappears, and the chromatic masses are divided by constriction into two or more portions which move apart. In a few cases the divided chromatic masses became associated with new nuclear membranes, but it is not clear whether this is always the case. Figures of this type are common in the portions of the thallus where active cytoplasmic movement has ceased. Inasmuch as there are frequently large amounts of reserve substances present at these stages, the question arises whether these presumably amitotic figures do not, in reality, represent typical mitotic figures in which the finer details of the achromatic figure are obscured by ergastic materials. On the other hand this type of division may represent the first stages in the degeneration of nuclei which have become superfluous.

The results of this study indicate that nuclear degeneration occurs in some cases, but no evidence has been found that it always occurs at the same stage. The phenomenon may also be exhibited after improper fixation, which may lead to erroneous conclusions regarding its frequency. Nothing was seen to indicate that the failure of nuclei to fuse in the zygospore necessarily leads to ultimate degeneration of the unfused nuclei. In certain species unfused nuclei appear to persist throughout the life cycle.

Both vegetative and sexual fusions of nuclei have been frequently reported in this group. The significance attached to this phenomenon seems largely a matter of interpretation. The obvious difficulties in distinguishing between late stages in nuclear fusion and early stages in nuclear division where an achromatic figure cannot be demonstrated are manifest. For this reason only those cases in which nuclei with membranes intact have been observed in the process of fusion have been regarded as having any biologic significance. The fusion process usually results in a marked increase in size of the fusion nucleus. It has never been possible to demonstrate any fundamental difference in the fusing nuclei, or to trace their possible parentage. From analogy with other forms, however, it seems reasonable that only nuclei of different genetic composition undergo karyogamy. Fusion takes place at different stages in the zygospore of the several species studied, and no evidence has been found of the reported vegetative karyogamies in the mycelial stages.

The results of many workers indicate that the nuclei of the vegetative

mycelium and the zygospores are not always of the same size or appearance at different stages. In regions where active cytoplasmic movements are going on the nuclei are usually larger with a well defined membrane and nuclear reticulum, whereas in regions where the growth rate is retarded or has ceased the nuclei tend to be contracted and the nuclear membrane difficult to demonstrate. This size difference which is correlated with cytoplasmic activity can be noted in both fused and unfused nuclei. Since these two phases are so characteristic it has seemed well to apply the terms "expanded nuclei" and "unexpanded nuclei" respectively to describe the two conditions. These noncomittal terms have been purposely selected since they imply no fundamental difference in the nuclear structure. The two phases grade into one another in transitional regions. The actual size of the nucleus varies greatly in different species. This change in nuclear size which is neither the result of karyogamy nor of nuclear reduction has not been clearly recognized in earlier investigations, and it is of considerable importance in the proper interpretation of nuclear behavior in the zygotes.

During meiosis the fusion nuclei are larger than the unfused nuclei and are much more prominent in the cell. In the meiotic divisions chromosomes are clearly delimited and are of considerable size. The chromosome number in those species in which it has been determined is 12 in the haploid condition. Clear meiotic figures have not been seen in all the species studied; but it has been possible, owing to the considerable size difference between fused and unfused nuclei, and by analogy from those species in which reduction division has been demonstrated, to locate the approximate point in the life cycle where this process takes place in all the species included here. The expanded and unexpanded nuclear phases referred to above must be taken into consideration when the criterion of nuclear volume is used to distinguish haploid from diploid nuclei, since both fused and unfused nuclei enter these characteristic phases.

An investigation of the cytoplasmic constituents of the thallus of these forms by the use of basic fixatives and cytoplasmic stains has revealed, in addition to the various reserve substances usually present, two types of structure which seem to fulfill the definition of mitochondria given by Newcomer (23). These bodies are preserved by bichromates between a pH range of 4.6 and 5.0. In rapidly growing hyphal tips and in the young progametangia they appear in the form of long, very thin threads. In the zygospores and chlamydospores, just prior to the formation of the prominent reserve substances, there are present very minute granules which exhibit the same staining reaction as these threads. In all probability these structures are homologous as indicated by their response to different reagents and by the intermediate forms sometimes encountered.

Numerous workers have reported the presence of mucorine crystals in the

older mycelium and zygospores of these forms. A survey of the literature on the question conveys the impression that these structures are always present at some stage or other in the life cycle. The present results indicate that under many conditions these crystals are not developed at all. Ling Young (17) has presented an extensive account of the formation and composition of these crystals and nothing can be added to his observations. The presence of amorphous bodies apparently concerned with the secretion of oil has been frequently pointed out. Keene (14) and Moreau (19) have discussed these bodies and speculated as to their origin. In general the observations of these two investigators on this category of ergastic substances is confirmed, although additional evidence indicating that the mitochondria may function in their formation is presented below. Keene's usage of the term "oil plastids" for these structures will be followed.

OBSERVATIONS

MUCOR GENEVENSIS Lendner. This homothallic species falls in the section *Hagemia* of the genus *Mucor* in the classification of Naumov (21). A number of species in this section have been investigated by various authors most of whom are in agreement concerning the nuclear behavior in the younger zygote stages. Dangeard (8) examined *Mucor fragilis* Tode, a species excluded by Naumov, and probably synonymous with *M. hiemalis* Wehmer, and observed nuclear fusions in the developing zygospore. The nuclei which failed to conjugate apparently disintegrated. In the azygospores he did not encounter any evidence of nuclear fusion or degeneration; the nuclei remaining unchanged throughout the development of the azygospores. Moreau (19) reports nuclear fusions, which he interprets as karyogamies, during the period when the exospore of the zygote is being deposited, in *M. sylvaticus* Hagem, *M. hiemalis* Wehmer and *M. genevensis* Lendner. Degeneration of unfused nuclei then occurs. Ling Young (17) working with *M. hiemalis* and *M. genevensis* corroborates Moreau's observations but does not extend them. None of these workers followed the nuclear condition through the dormant period of the zygospore, nor did they observe germination of the zygospore.

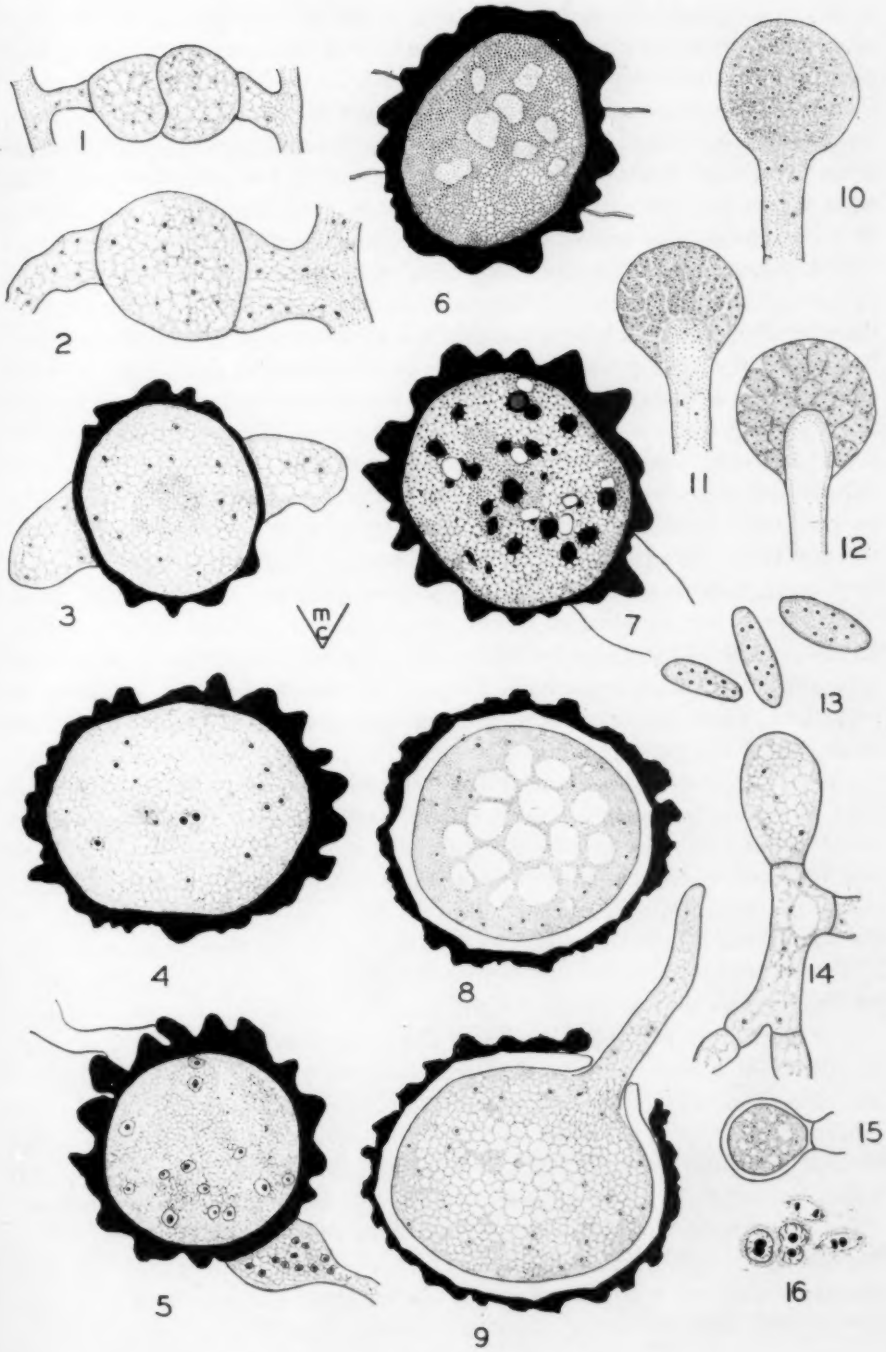
The present study confirms, in general, the observations of these workers on the early stages of zygospore development. The progametangial initials are multinucleate and mitotic divisions are in evidence as they increase in size (fig. 1). The number of nuclei in the young gametangia is not large. Nuclear division ceases as the coenozygote is formed, and perhaps fifty nuclei are present in the average coenozygote (fig. 2). These nuclei are arranged indiscriminately in the cell, and as the exospore matures they show a paired arrangement and a marked increase in size (fig. 4). As the exospore assumes its mature form, very distinct nuclear fusions are in progress (fig. 61). In all the material examined the great majority of nuclei in the cell

fused in pairs, and only an occasional unfused nucleus could be discerned in zygospores 4 days old (figs. 5, 62). These fusion nuclei persist in the expanded state for several days during which time oil plastids become very numerous in the zygospore, and the cytoplasm becomes quite dense. On about the sixth day of development the endospore is differentiated and almost simultaneously with the appearance of this structure a distinct change in the size and number of nuclei in the zygospore takes place (fig. 8). In place of the few large expanded fusion nuclei previously in evidence, there are now apparent about twice as many smaller nuclei, which remain in the expanded state for only a short time and then rapidly sink into the unexpanded phase with the onset of dormancy in the zygote. From analogy with the situation described below in *Absidia spinosa*, where the nuclear behavior is similar to that of *M. genevensis* and where, apparently, the meiotic divisions occur at this stage, it appears reasonable that this change in nuclear size and appearance is the result of a reduction division of the fusion nuclei. By the end of the eighth day after the formation of the coenozygote, most of the zygospores have entered the dormant condition. The resting period persists for about 4 months and no further change in the nuclear condition can be seen. The cytoplasm gradually contracts into the peripheral regions of the spore and the oil plastids are very prominent, tending to obscure all the other cell constituents.

Zygospores of this species are capable of germination approximately four months after their formation and remain viable until the end of the seventh month. At the onset of germination the oil reserve disappears, the cytoplasm becomes vacuolate and rapid mitotic divisions take place which differ in no way from those seen in the mycelium and progametangia. The zygospore walls are soon ruptured and a thick germ tube is pushed out (fig. 9). At this stage the exospore wall becomes quite translucent and germinating zygospores can be easily distinguished from those still in the dormant con-

Explanation of figures 1–16, Mucor genevensis

FIG. 1. Progametangia in contact (Flemming's; Haematoxylin). ×660. FIG. 2. Coenozygote 7 hours old (Flemming's; Haematoxylin). ×660. FIG. 3. Zygospore 15 hours old. Dark region in center may represent coenocentrum (Flemming's; Cahal-Brozek). ×660. FIG. 4. Zygospore 2 days old; fused and unfused nuclei present; central nuclei undergoing syngamy (Chamberlain's; Haematoxylin). ×660. FIG. 5 Zygospore 5 days old with fusion nuclei in expanded phase (Gilson's; Feulgen). ×660. FIG. 6. Zygospore 4 days old showing mitochondria and formation of oil plastids (Zirkle's; Haematoxylin). ×660. FIG. 7. Zygospore 5 days old, showing chondriome and oil plastids; the clear areas probably represent nuclear vacuoles from which the chromatin has been dissolved (Z.R.C.; Bensly-Cowdry). ×660. FIG. 8. Dormant zygospore; nuclei in unexpanded phase (S.M.C.; Feulgen). ×660. FIG. 9. Germinating zygospore 4 months old (Flemming's; Triple). ×660. FIGS. 10–12. Sporangium development (Flemming's; Haematoxylin). ×660. FIG. 13. Mature sporangiospores; nuclei unexpanded (F.A.A.; Buffer). ×1000. FIG. 14. Hyphal bodies and young terminal chlamydospore (Flemming's; Cahal-Brozek). ×660. FIG. 15. Terminal chlamydospore 6 days old (F.A.A.; Buffer). ×660. FIG. 16. Stages in nuclear fusion and mitotic division in zygospore 3 days old (Flemming's; Haematoxylin). ×1350.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dition. The germ tube develops into a single unbranched sporangiophore terminated by a germ sporangium. The sporangiospores of this germ sporangium all give rise to homothallic mycelia.

Particular attention has been given to the nature of the chondriome in this species. In the hyphal tips of the mycelium and in developing sporangia long, extremely delicate, thread-like mitochondria are arranged in a loose weft in the regions of active cytoplasmic movement. As the cytoplasmic activity ceases in the older portions of the mycelium, these structures are no longer discernible, but minute granules can be demonstrated by the same fixing and staining methods. These mitochondria become particularly abundant in the regions where accumulation of reserve products takes place. They are very prominent in developing chlamydospores and in the younger stages of the coenozygotes, where they are arranged in a regular reticulum. They persist in this condition until the appearance of the oil plastids which seem to arise by a coalescence of these granules (figs. 6, 7, 65). The oil plastids exhibit the same staining reactions as the mitochondria, but this might be attributed to the masking effect of many mitochondria aggregated over the surface of the plastids. In the stages where the oil plastids are fully developed, mitochondria can no longer be demonstrated as distinct units. They have either disappeared or have been incorporated into the oil plastids. In the germinating zygospores, where the oil plastids and most of the reserve substances have disappeared the granular mitochondria have not been observed, but the thread-like forms are again apparent in the active cytoplasm at the tip of the germ tube.

The formation of the sporangiophores and sporangia of this species follows in the early stages the same course which Moreau (19) describes for *Mucor spinescens*. The sporangiophores are usually unbranched, and at their tips terminal swellings develop, into which a considerable amount of cytoplasm is carried. Mitotic nuclear divisions take place with great rapidity in the sporangial fundament (fig. 10). As the fundament reaches its maximum size the cytoplasm becomes differentiated into a peripheral sporogenous layer which contains most of the nuclei and an internal more or less sterile region which ultimately becomes the site of the columella. This stage is of short duration and then numerous flattened vacuoles appear in the fertile zone and divide the cytoplasm into many multinucleate blocks (fig. 11). These blocks may contain as many as 15–20 nuclei. At the same time a broad vacuole is delimited on the site which the columella membrane is to occupy. These vacuoles are filled with a refractive hyaline intersporal substance, which appears to function in the deposition of the sporangiospore walls and the columella membrane. After the appearance of the intersporal substance the multinucleate cytoplasmic segments contract somewhat and without further subdivision assume the characteristic elongated shape of the spo-

rangiospores. They then acquire a spore wall, and at the same time, a heavy wall which becomes the columella is deposited around the lower edge of the broad vacuole which separates the fertile from the sterile region (fig. 12). It must be emphatically stated that the columella does not arise by the upward burgeoning of the septum which is usually formed below the sporangial fundament. The few nuclei present in the columella soon revert from the expanded to the unexpanded phases and amitotic divisions may occur. The cytoplasm becomes very vacuolate and soon contracts against the walls of the sporangiophore, or becomes incorporated into chlamydospores which in some strains of this fungus develop luxuriantly in the sporangiophores. It will be noted that the process of sporangiospore formation in this species differs from that described by Moreau in *M. spinescens* in that the subdivision of the sporogenous cytoplasm ceases at an earlier stage, and this cytoplasm does not become arranged into cords before the spore initials are delimited. This is presumably a more primitive condition than that in *M. spinescens* since reduction in spore size and nuclear number is not carried so far (fig. 13).

Chlamydospores are very abundant in this species and in some strains the submerged mycelium may be almost completely transformed into these bodies. In newly formed chlamydospores (fig. 14) the nuclei are generally in the expanded condition and very occasionally mitotic divisions can be seen, but the nuclei enter the unexpanded phase as soon as the reserve substances begin to accumulate. Oil plastids of the same type as those seen in the zygospores are found in the chlamydospores and the same relationship between oil plastids and mitochondria has been observed.

MUCOR HIEMALIS Wehmer. This is a heterothallic species very closely related to *Mucor genevensis*, previously investigated cytologically by Moreau (19) and Ling Young (17). Both these authors find that in all stages investigated by them the nuclear behavior is completely similar in both species. In the strain of *M. hiemalis* used here all the sexual and asexual stages investigated were entirely similar to the corresponding stages in *M. genevensis* as far as nuclear behavior was concerned, and the species could not be distinguished cytologically.

The problem of sex segregation in this form presents certain peculiar aspects. The sporangiospores of any given germ sporangium produce, upon germination, mycelium which is entirely of one sex or the other. To date no sporangia have been found which contain spores of both sexes. In other words, segregation of sex is complete at the time of reduction division. This is unusual in that no plausible explanation has presented itself why only spores of one sex should persist at the expense of spores of the other sex in any given germ sporangium. The conditions affecting this phenomenon are as yet problematical and further investigation is necessary.

PARASITELLA SIMPLEX Bainier. This species, encountered as a parasite upon various genera of the Mucorales, resembles very closely some of the species in the *Hagemia* section of *Mucor*. Taxonomically it is particularly close to *M. hiemalis* Wehmer. From these it can be distinguished by its habit of forming curious galls or "sikyospores" upon the hyphae of species of the Mucorales with which it comes in contact. Burgeff (5) and Satina and Blakeslee (24) have investigated this phenomenon in detail. Burgeff has also carried out a cytological examination of the nuclear behavior in these galls. As far as can be determined, the nuclear condition has not been followed through the development of the zygospore of *Parasitella*, although Burgeff has discussed the development of the zygospore from a morphological point of view.

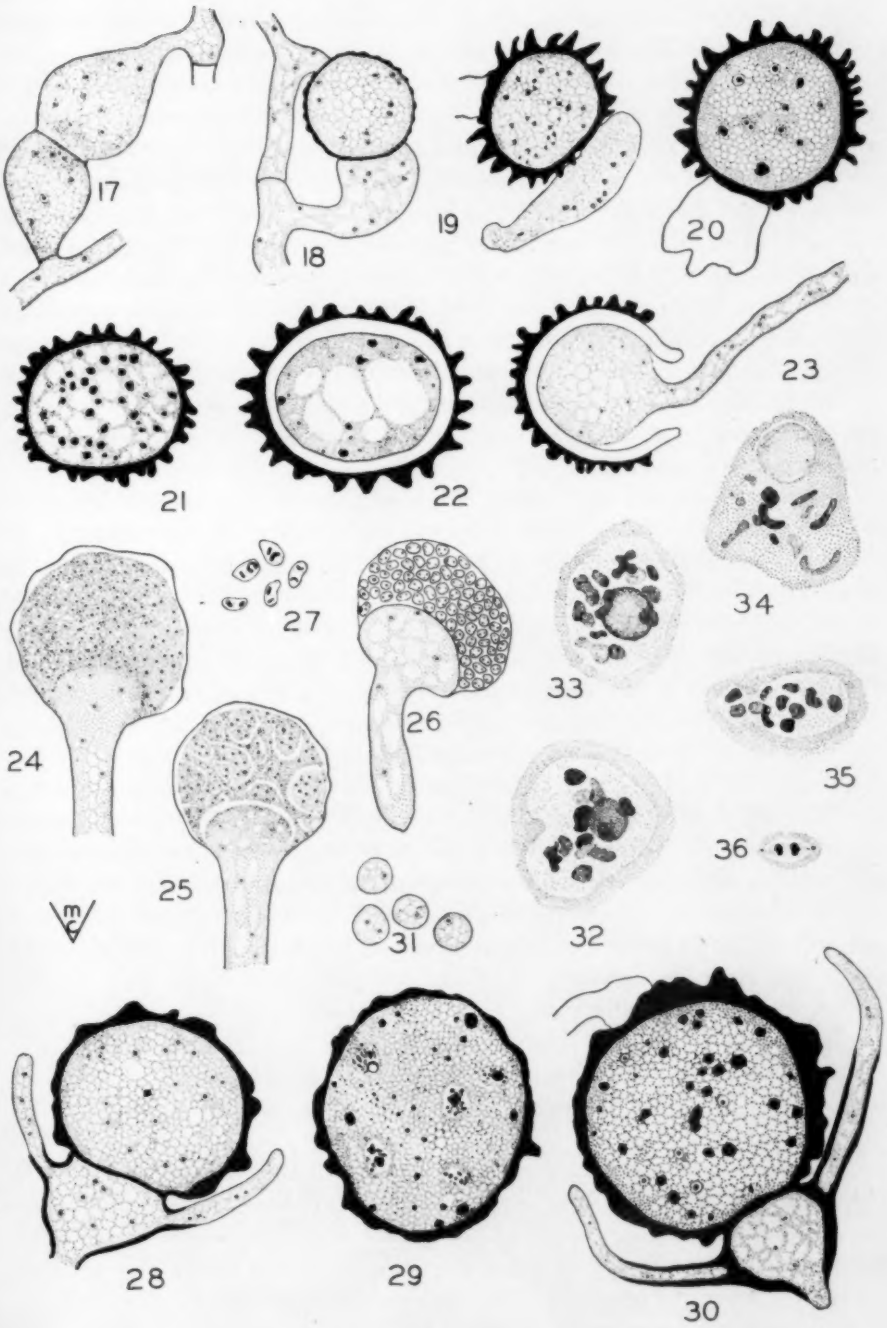
In our material of *Parasitella* zygospores were formed in small numbers, and were in all respects identical to those described by Burgeff, with the exception that in our cultures no spores were found bearing the *Absidia*-like outgrowths from their suspensors which Burgeff has illustrated for some of his material.

The nuclear condition in this strain of *Parasitella* differs in no way from that previously described in *M. genevensis* and *M. hiemalis*. Germination of the zygospores proceeds along the same pattern and occurs within the same time range. *Parasitella simplex* differs in only a few minor morphological details from *M. hiemalis* and cannot be differentiated, in our strains at least, on a cytological basis.

ZYGORHYNCHUS DANGEARDI Moreau. A detailed review of all the literature pertaining to *Zygorhynchus* will not be attempted, since Green (9) has presented a comprehensive account of the progress of this work up to 1927. The

Explanation of figures 17–27, Zygorhynchus dangeardi; and of figures 28–36, Absidia spinosa

FIG. 17. Progametangia in contact (Chamberlain's; Cahal-Brozek). ×660. FIG. 18. Zygospore 8 hours old (Flemming's; Haematoxylin). ×660. FIG. 19. Zygospore 15 hours old, some nuclei degenerating (Flemming's; Triple). ×660. FIG. 20. Zygospore 3 days old; fusion nuclei in expanded phase; oil plastids developing (Flemming's; Haematoxylin). ×660. FIG. 21. Zygospore 6 days old; cytoplasm becoming vacuolate and oil plastids numerous (Craf; Haematoxylin). ×660. FIG. 22. Dormant zygospore; nuclei unexpanded (F.A.A.; Buffer). ×660. FIG. 23. Germinating zygospore 78 days old; mitotic divisions in germ tube (Flemming's; Triple). ×660. FIGS. 24–26. Sporangium development (Flemming's; Triple). ×660. FIG. 27. Sporangiospores 2 weeks old; nuclei unexpanded (F.A.A.; Buffer). ×1000. FIG. 28. Zygospore 16 hours old, unfused nuclei in expanded phase (Flemming's; Feulgen). ×660. FIG. 29. Zygospore 7 days old; 5 fusion nuclei in prophase of first meiotic division; oil plastids developing (Craf; Haematoxylin). ×660. FIG. 30. Zygospore 3 days old; fusion nuclei in expanded phase; oil plastids numerous (F.A.A.; Cahal-Brozek). ×660. FIG. 31. Sporangiospores (F.A.A.; Buffer). ×1000. FIGS. 32–34. Prophase stages of first meiotic division in zygospores 7 days old; the large nucleolus-like bodies probably represent developing oil plastids (Craf; Haematoxylin). ×3100. FIG. 35. Metaphase of first meiotic division (Craf; Haematoxylin). ×3100. FIG. 36. Anaphase of mitotic division in vegetative mycelium (Craf; Haematoxylin). ×3100.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only important contribution which has appeared since that time is Ling Young's (17) account of the nuclear behavior in *Z. moelleri*, *Z. macrocarpus*, and *Z. dangeardi* in 1930. No attention will be given to the morphological development of the fungus, this having been adequately described by Atkinson (1), Blakeslee (3), Green (9), and other workers.

The nuclear behavior in *Z. dangeardi* has been discussed by Moreau (19). He points out that it differs from its close relative, *Z. moelleri* Vuill., in that the number of fusion nuclei in the zygospore is greatly reduced. In some cases there are only four fusion nuclei present in the zygospore prior to the onset of the dormant condition. Nuclear fusion also takes place at a later stage in zygospore development than in *Z. moelleri* Vuill. In *Z. macrocarpus* Ling Young, which according to its author (17) resembles *Z. dangeardi* very closely in its morphological characters, the nuclear behavior follows the same pattern as that in *Z. moelleri;* that is to say the number of fusion nuclei are not particularly reduced as in *Z. dangeardi.*

In the zygophores and young progametangia of the present strain of *Z. dangeardi*, expanded, unfused nuclei are present in fair numbers. It should be emphasized, however, that the cytoplasm of all the species of *Zygorhynchus* does not possess the extremely large number of very minute nuclei which are characteristic of such genera as *Rhizopus*, *Phycomyces*, and *Sporodinia*. The nuclei are also somewhat larger in proportion to the thallus size than in the aforementioned genera. As the progametangia develop, and the suspensors are delimited by the typical cleavage furrow which develops in the form of a closing diaphragm, sporadic mitotic divisions are apparent. The nuclei in these young sex organs do not, however, become proportionately more numerous than in the zygophores (fig. 17). In the young coenozygote the number of nuclei is not large, and there is no evidence that any nuclear degeneration has yet occurred. As the exospore matures, nuclei may be observed which are fused, associated in pairs, or unfused. All nuclei are still in the expanded condition. Nuclear fusions occur until about the sixth day after the appearance of the exospore when apparently all the nuclei which entered the young coenozygote have fused in pairs (fig. 20). No indication of the degeneration of any nuclei has been observed in this species. The number of fusion nuclei present varies with the size of the spore, but is never large. On the average 10–12 fusion nuclei are present in most zygospores at this time.

Oil plastids are developed rather sparingly in this species, and arise in the same manner as that described for *Mucor genevensis* (fig. 21). They persist through the resting period of the zygote and disappear shortly before germination. The zygospores enter the dormant period at the end of about eight days when the hyaline endospore becomes fully differentiated. Reduction division has presumably occurred just prior to this stage, for in zygo-

spores 10–15 days old approximately 40–50 small, apparently unfused nuclei are present. These soon change from the expanded to the unexpanded phase and persist in this condition until the time of germination (fig. 22).

As far as can be determined the germination process has not been previously observed in *Z. dangeardi*. The zygospores are capable of germination after a resting period of about 40 days and retain their viability for at least six months. At the expiration of the rest period the cytoplasm becomes very foamy, the oil plastids disappear and the nuclei resume the expanded condition and undergo mitosis. Shortly the zygospore walls are ruptured and one or more branching germ tubes are put forth (fig. 23). These germ tubes, like those of *Blakeslea trispora*, develop an extensive nutritive mycelium before any sporangiophores are produced. All attempts to induce these germ tubes to develop directly into sporangiophores have failed. The spores of the germ sporangium are all homothallic.

Development of the sporangiophore and sporangium in this genus is in almost all respects similar to that described in *Mucor genevensis* (figs. 24–26). The number of nuclei per sporangiospore is smaller (fig. 27), usually 3–5 nuclei being incorporated in each spore. In some cases the sporangial membrane becomes very finely echinulate with spicule-like projections of calcium oxalate arising from its outer surface. Occasionally a sporangium was observed in which segmentation of the cytoplasm into individual spores had not taken place, and the sporangium had matured with the cytoplasm remaining as a single unit. The columella of this type of sporangium usually is not developed, in which case the resulting structure resembled an azgyospore save that the exospore and suspensor were not well developed. This phenomenon illustrates the probable homology of the sexual and asexual generations, and possibly indicates the direction in which the evolution of the asexual sporangium from the sexual zygospore proceeded. Chlamydospores, although not frequent in this species, are developed in the usual manner when present.

ZYGORHYNCHUS MOELLERI Vuill. The development of this species has been investigated at length by Green (9), Moreau (19), Gruber (10), Atkinson (1), Ling Young (17) and others. Moreau points out that in *Z. moelleri* reduction in the number of fusion nuclei is not so pronounced as in *Z. dangeardi*, and he feels that reduction division occurs at the time of zygospore germination although he was not able to demonstrate this process. Ling Young's (17) results are in accord with those of Moreau. Green (9) was unable to complete a satisfactory cytological study of nuclear behavior in the zygospore because of the small size of the nuclei.

In the strain of *Z. moelleri* used here, there were usually more fusion nuclei present in proportion to the size of the zygospore than were observed

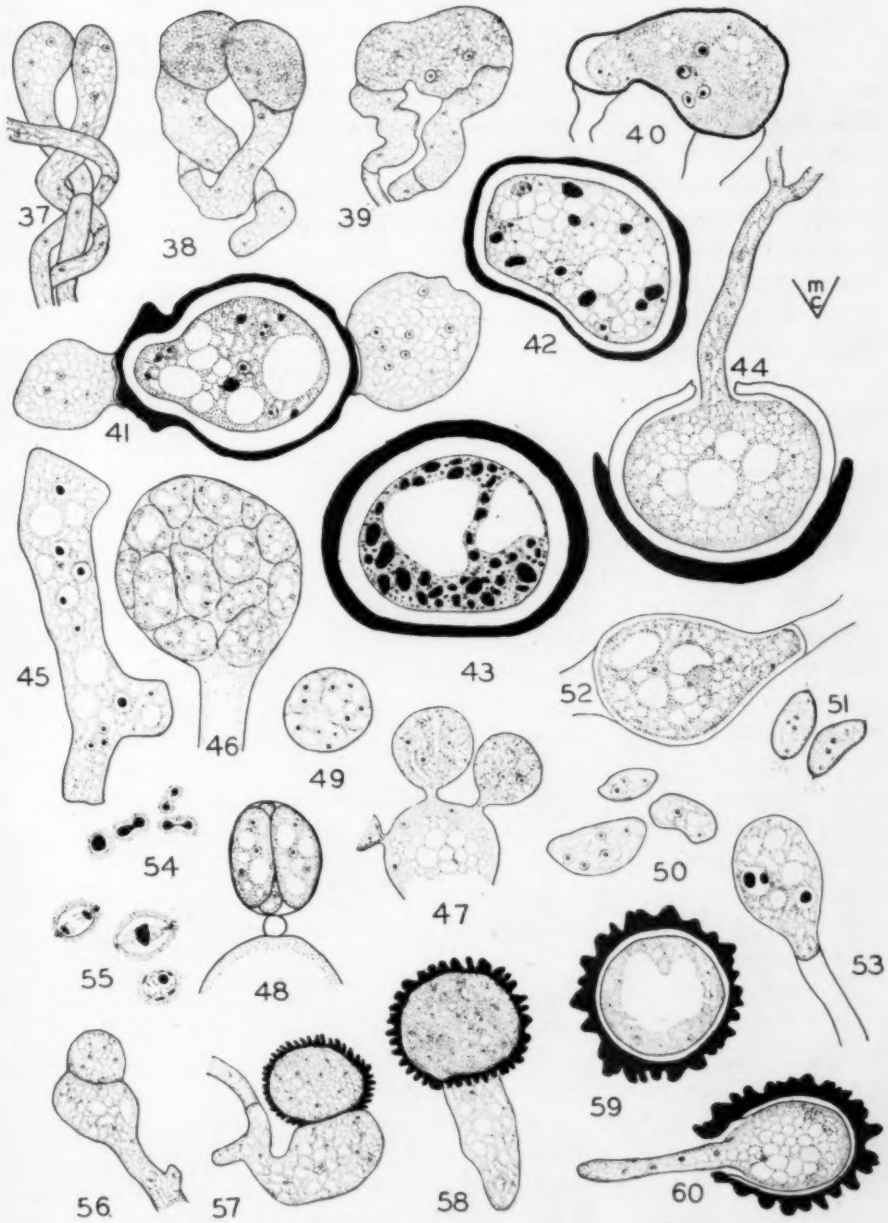
at corresponding stages in *Z. dangeardi*, but the difference is not consistent and certainly does not warrant specific distinction. The remaining stages in the life history were similar in all details to those already described in *Z. dangeardi*. As far as could be determined from nuclear number and volume, reduction division occurs prior to the dormant period, and no evidence was encountered to support Moreau's hypothesis that this process takes place during the germination of the zygote.

ZYGORHYNCHUS VUILLEMINII Namysl. The nuclear behavior in this species has not been previously investigated thoroughly, although Namyslowski (20) has discussed at length the conditions necessary for gametic reproduction. Ling Young (17) in the course of a general discussion of the nuclear behavior in the Mucorales mentions that the nuclear condition in this species is similar to that of *Z. moelleri*. In the material studied here the nuclear condition differed in no appreciable way from that in *Z. moelleri*. Except for minor differences in colony color and zygospore ornamentation there are apparently no reliable criteria for separating them. Since these characters appear somewhat variable the taxonomic validity of the two species may be questioned.

ZYGORHYNCHUS VUILLEMINII var. AGAMUS Namysl. This variety of *Zygorhynchus vuilleminii* is a predominately apandrous strain in which the majority of zygospores develop without the presence of the smaller or male gametangium. In this material only a very occasional zygospore could be

Explanation of figures 37–55, Blakeslea trispora; and of figures 56–60, Zygorhynchus vuilleminii var. agamus

FIG. 37. Progametangial initials in contact (Flemming's; Haematoxylin). ×415. FIG. 38. Gametangia with intervening wall dissolved (Flemming's; Haematoxylin). ×415. FIG. 39. Young coenozygote; nuclei enlarging prior to fusion (Flemming's; Haematoxylin). ×415. FIG. 40. Older coenozygote showing several paired nuclei, and several degenerating nuclei (F.A.A.; Cahal-Brozek). ×415. FIG. 41. Zygospore with fusion nuclei in expanded phase; cytoplasm becoming vacuolate (Chamberlain's; Triple). ×415. FIG. 42. Zygospore 6 days old with fusion nuclei and oil plastids becoming numerous (F.A.A.; Buffer). ×415. FIG. 43. Zygospore in dormant condition; nuclei unexpanded (F.A.A.; Buffer). ×415. FIG. 44. Germinating zygospore (Gilson's; Feulgen). ×415. FIG. 45. Hyphal body with vegetative nuclei in expanded phase (Flemming's; Haematoxylin). ×750. FIG. 46. Sporangium without columella; spores forming (S.M.C.; Feulgen). ×415. FIGS. 47–49. Sporangiola formation (Flemming's; Triple). ×415. FIG. 50. Young sporangiophores; nuclei expanded (F.A.A.; Buffer). ×415. FIG. 51. Mature sporangiospores; nuclei unexpanded (F.A.A.; Buffer). ×415. FIG. 52. Intercalary chlamydospore (F.A.A.; Buffer). ×415. FIG. 53. Young terminal chlamydospore; nuclei expanded; 1 nucleus with 2 central bodies (Flemming's; Haematoxylin). ×600. FIG. 54. Amitotic divisions in columella of sporangiophore, (Flemming's; Feulgen). ×1350. FIG. 55. Mitotic prophase, metaphase and anaphase in hyphae (Flemming's; Triple). ×1350. FIG. 56. Azygospore forming (Chamberlain's; Triple). ×660. FIG. 57. Azygospore 1 day old (Flemming's; Triple). ×660. FIG. 58. Azygospore 7 days old; endospore not yet formed (Chamberlain's; Cahal-Brozek). ×660. FIG. 59. Dormant azygospore (Gilson's; Feulgen). ×660. FIG. 60. Germinating azygospore (Flemming's; Triple). ×660.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found which possessed two authentic suspensors. When undoubted sexual reproduction occurred the nuclear condition followed the same pattern as in *Z. vuilleminii*. In all the studies made on these azygospores the material was carefully examined before fixation and any zygospores were rejected, in order that a more nearly exact conception of nuclear behavior in a typically azygous Mucor might be obtained.

The early stages in the formation of the azygospores closely resembled those encountered in the zygospores. The larger progametangium (fig. 56) grows out from the zygosporic hyphae and quite frequently manifests a weak zygotrophism towards the portion of the zygophore where the smaller or oppositely sexed progametangium would normally be developed. This would indicate that the apandrous condition is a degenerate one and that in certain cases the localization of opposed sexual tendencies has not been completely lost.

The nuclear situation in the larger progametangium is the same as that in the corresponding gametangium of the normal strain. The nuclei through all the developmental stages remain in the expanded phase. When the gametangium has reached its maximum size a heavy, characteristically decorated exospore wall is laid down and the cytoplasm becomes vacuolate, whereupon oil begins to accumulate in the vacuole (figs. 57, 58). The prominent oil plastids seen in the zygotes of other species investigated have not been encountered in the azygospores, although they are developed in the occasional zygospores that are formed on this strain. Inasmuch as no nuclear fusions of any type have been encountered in these azygospores it may be possible that the presence of oil plastids is in some way correlated with the presence of nuclear fusions in the zygospore, since they are apparent in all species where nuclear fusions have been demonstrated, while they occur very rarely in the present variety and in species such as *Sporodinia grandis* where nuclear fusions have not been observed. The fact that these plastids were observed in conjunction with the meiotic nuclei in *Absidia spinosa* is also significant in this connection.

The azygospore enters the dormant period with the appearance of the hyaline endospore when about eight days old (fig. 59). The resting period lasts approximately 30 days. Germination takes place in the same manner described for the zygospores of *Z. dangeardi* (fig. 60). Since no nuclear fusion has ever been observed in this form and since, presumably, nuclei of only one sex are present in the azygospore, germination appears to be almost certainly apomictic. As pointed out above, the infrequent presence of a true zygospore in this strain; the fact that all intergrades between typical sexual reproduction and the apomictic development of the azygospores can be demonstrated; and Namyslowski's report of an occasional culture of this strain in which gametic reproduction is completely suppressed; all point to

the conclusions that in this case the absence of a true sexual reaction is an advanced condition, and that the presence of the sexual reaction is, in *Zygorhynchus* at least, the primitive condition. Attempts have been made to induce a sexual response between this apandrous strain of *Z. vuilleminii* and sexually potent strains of heterothallic species such as *Mucor hiemalis* with very indifferent success. All the evidence indicates that this strain is practically impotent. This might be further interpreted as proof of the contention that, in the Mucorales, heterothallism is the primitive condition, and the assumption of homothallism, which may ultimately be followed by the development of apomictic and finally sexually sterile races, represents an advanced condition.

ABSIDIA SPINOSA Lendner. This homothallic species has been investigated cytologically only by Moreau (19) and Ling Young (17). Moreau reports the nuclear condition as similar to that described by him in *Mucor genevensis*, and Ling Young corroborates him.

The zygospores in this material were very markedly heterogamic, and upon the larger of the two suspensors several long, circinately curved appendages are produced. As the progametangia develop, they push the zygosporic filaments apart, and very shortly after the initial contact is established the suspensors are delimited from the progametangia by transverse walls. These walls in development simulate a closing diaphragm and are formed by a circular cleavage furrow which develops inward from the periphery of the progametangial cell. Since the progametangia are minute at the time when the suspensors are separated from them, it seems probable that these separating walls do not close until a later stage in development. Shortly after the suspensors are delimited, and before the formation of the coenozygote, the appendages of the larger suspensor arise as buds upon its surface. These buds receive nuclei from the suspensors and grow very rapidly, ultimately attaining a length several times that of the zygospore, which is enclosed within these structures. Mitoses of the usual type take place within the developing appendages, but as they reach maturity and cytoplasmic activity ceases amitotic divisions become evident. The appendage walls become much thickened but an open connection is apparently always maintained with the interior of the suspensor (fig. 28). In mature appendages the cytoplasm appears to be evacuated through this opening, back into the suspensor leaving only the rigid appendage wall present. Regeneration experiments, in which the appendages were dissected from the suspensors and isolated on nutrient media, indicate that these appendages are not capable of renewed growth after the heavy wall has been deposited, thus suggesting that the cytoplasm has degenerated or been completely removed. This may be further indication that amitotic nuclear divisions are characteristic of degenerating cytoplasm.

As the cytoplasm of the gametangia mingles to form the coenozygote, the nuclei present in the fusion cell enlarge in size, but no further nuclear divisions are evident; hence the number of nuclei in the coenozygote is not large in comparison with such a form as *Rhizopus nigricans*, in which very rapid nuclear divisions take place at this stage. These unfused nuclei remain in this slightly enlarged expanded state until the exospore wall has been deposited (fig. 28), but a marked association of the nuclei in pairs such as is evident in *Blakeslea trispora* (fig. 40) has not been seen. Soon after the deposition of the exospore wall, which generally occurs in coenozygotes 15–20 hours old, nuclear fusion takes place and as far as could be determined, all the nuclei present in the cell fuse in pairs. No evidence whatever has been seen of the degeneration of unfused nuclei. The fusion nuclei persist in the expanded state until the zygospore is about six days old (fig. 30), during which time vacuoles and oil plastids begin to make their appearance in the cytoplasm.

The best evidence of the nature of the meiotic divisions in the Mucorales has been obtained from studies of division figures in this species. In zygospores 6 to 7 days old nuclear figures which show all the characteristics of meiotic prophases and metaphases have been observed. Figures 29, 63 illustrate a zygospore in which a metaphase and three prophases are visible. Figures 32–35 illustrate the individual stages. It will be noted that these meiotic nuclei are many times the size of the usual vegetative nuclei and that the chromosomes are clearly visible. Since the chromosomes are so markedly enlarged beyond the size of the vegetative nucleus, and since in the nuclei shown in figures 32–35 no indication of any achromatic spindle could be discerned, these figures have been tentatively identified as late diakinesis stages. The large nucleolus-like structure present in these figures proves particularly difficult to interpret. The similarity of these structures to the developing oil plastids scattered through the zygospore at this stage raises grave doubts of the possible interpretation of them as nucleoli. As yet no fundamental difference between these structures and the young oil plastids have been demonstrated. The arrangement of the chromosomes in a plate suggests that figure 35 is a prometaphase in which the spindle has not been fully developed, while the lack of a nucleolus-like body in this figure might give further support to this contention. However, as pointed out above, too much significance cannot at present be attached to the presence or absence within the nuclear vacuole of these putative nucleoli. In the light of present evidence they appear to be oil plastids which have arisen within the nucleus. Their presence in these figures and their similarity to the other oil plastids in the cell gives added credence to Baird's contention (2) that these structures which, in *Phycomyces blakesleeanus*, he terms "reserve bodies," arise within the nuclei and are filled with stainable substances elaborated by the

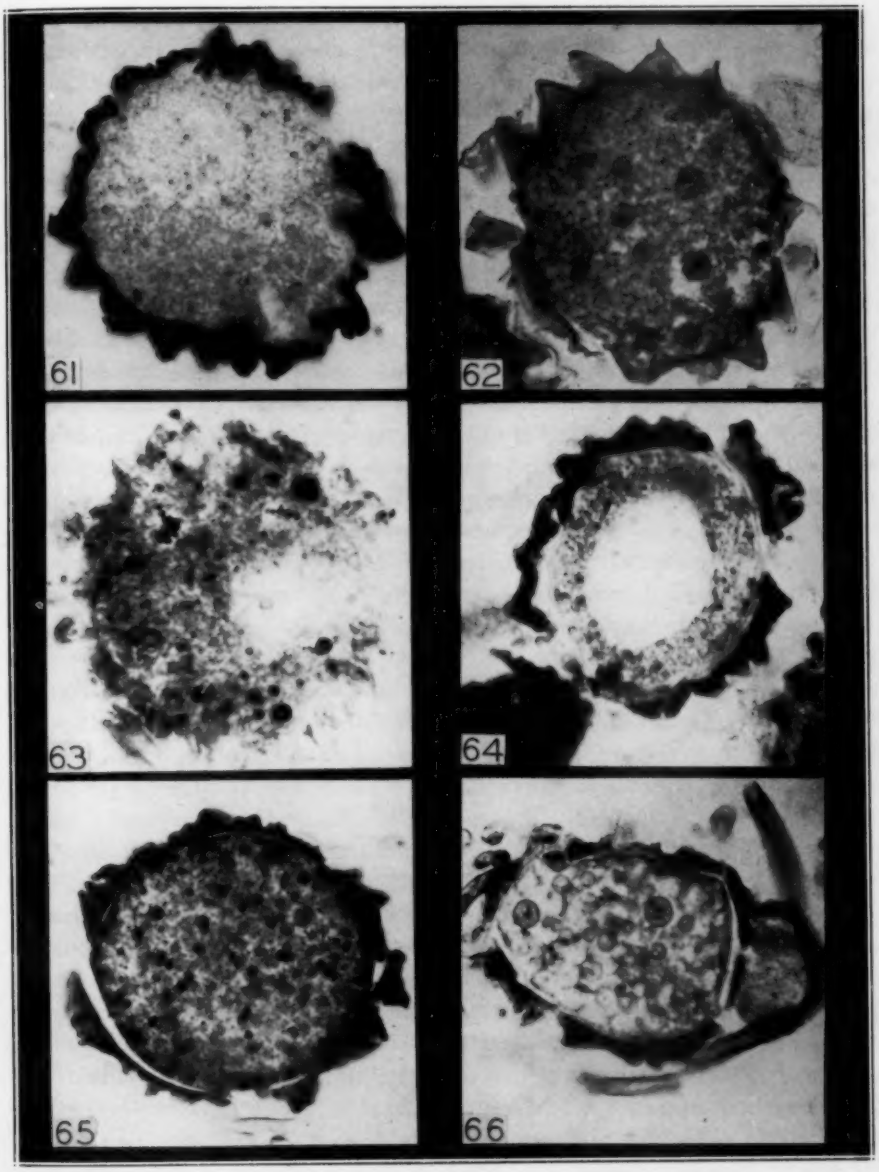

FIG. 61. *Mucor genevensis.* Unfused nuclei in zygospore 3 days old (Gilson's; Haematoxylin). ×700. FIG. 62. *Mucor genevensis.* Fusion nuclei in zygospore 6 days old (Flemming's; Triple). ×700. FIG. 63. *Absidia spinosa.* Prophases of first meiotic division in zygospore 7 days old; oil plastids beginning to accumulate (Craf; Haematoxylin). ×700. FIG. 64. *Absidia spinosa.* Dormant zygospore; nuclei in unexpanded phase (S.M.C.; Feulgen). ×700. FIG. 65. *Absidia spinosa.* Developing oil plastids in zygospore 9 days old; the cloudy appearance is caused by numerous mitochondria scattered in the cytoplasm (Zirkle's; Haematoxylin). ×700. FIG. 66. *Absidia spinosa.* Oil plastids in zygospore 12 days old; the chromosome-like bodies in the plastids are probably fixation artifacts (Craf; Buffer). ×700.

latter. If this is the correct interpretation and these structures merely represent young oil plastids, then the meiotic nuclei must be considered as anucleolate. Further investigation will be necessary to evaluate this supposition.

The chromosome number in all figures where it could be determined was consistently 12. Inasmuch as these figures have been interpreted as prophases of the first meiotic division the somatic chromosome number in *Absidia spinosa* is probably 12. This is significant in the light of Burgeff's (4) remarks concerning the chromosome number in *Phycomyces blakesleeanus*. In division figures which he interpreted as anaphases of the first meiotic division he estimated 24 chromosomes to be present. It is a point of interest that these two species should correspond in their chromosome numbers.

Subsequent stages in the meiotic division have as yet not been clearly demonstrated. Shortly after the stage in zygospore development, where these division figures were found, has been reached, the large fusion nuclei disappear, and about twice the number of smaller apparently haploid nuclei are present (fig. 64). This is the same situation which has been found in the species discussed above and in *Blakeslea trispora*, where as the zygospore enters the dormant period apparently unfused, unexpanded nuclei make their appearance. From these circumstances it has been inferred that the meiotic divisions occur very rapidly just prior to the time when the zygospore enters the dormant condition. At this stage the oil plastids become very numerous and tend to obscure most of the cytological details (figs. 65, 66). The subsequent development and germination of the zygospore of *A. spinosa* differs in no essential from that already described in *Mucor genevensis*.

Germination of the zygospore takes place after a dormant period of 3–4 months. The germ tube takes the form of an arching stolon upon which a single, or occasionally several fasicles, of sporangia are born. This primary stolon will, if placed upon a nutrient medium, develop rhizoids at its point of contact with the substratum, and thus build up an extensive mycelium before the spores in the germ sporangia are shed. The sporangiospores produced in these germ sporangia are all homothallic. The development of the asexual sporangia is similar to that described in the other forms studied here, save that the initial cytoplasmic segmentation is carried even further, so that many uninucleate sporangiospores are produced. In fact in this strain of *A. spinosa* uninucleated spores appear to be the rule rather than the exception, as they are in most other mucors. It will be noted that the nuclear behavior in *A. spinosa* varies in many respects from that which will be reported in a subsequent paper for *Absidia glauca* and *Tieghemella coerulea*, two species previously considered very closely related to *A. spinosa*.

BLAKESLEA TRISPORA Thaxter. Thaxter (28) has described the develop-

ment of the asexual fructifications of this species, and placed it in the Choanephoraceae. Weber and Wolf (29) have demonstrated the heterothallic nature of the species, and discussed the formation of the zygosporic stage. No cytological investigations have been carried out on this form although Wolf (30) has made a partial study of the nuclear condition in the closely related *Choanephora cucurbitarum*.

The nucleus of *Blakeslea* is larger and more conspicuous than in any of the other Mucorales included in this study. In the expanded condition it may reach in some stages a diameter of 4 microns (fig. 53). The number of nuclei in the thallus is fewer than in the less highly specialized species of this order, which probably indicates an advance towards the condition encountered in the higher fungi. In mitosis the achromatic spindle is well developed (fig. 55), but individual chromosomes have not been distinguished.

The formation of the sporangium takes place in much the same manner described by Harper (11) for *Sporodinia*, except that a columella is not regularly formed. The tip of a sporangiophore swells, assuming a more or less spherical shape. The cytoplasm of this sporangial fundament becomes very dense and many nuclei in the expanded condition accumulate within it. Most of these nuclei arise by mitotic divisions from nuclei already present in the fundament. No particular zonation of the nuclei such as is common in the sporangial fundament of most mucors has been observed here. When the sporangium has assumed its maximum size, numerous narrow vacuoles cut the protoplasm into polyhedral multinucleate segments. The vacuoles then become filled with a substance having a glassy, homogeneous appearance. Shortly thereafter the multinucleate segments begin to round up and develop a heavy, dark, longitudinally striate wall (fig. 46). At the poles of each spore a group of fine hair-like appendages radiate outwards from a small cap-like mass on the surface of the spore (fig. 51). These appendages could only be distinguished in mature spores freed from the sporangial wall and could never be demonstrated upon immature spore initials within the sporangium. Consequently their origin remains obscure, although Thaxter (28) feels that they are formed from the intersporal substance.

When a columella is developed in the sporangium, it is cut out by vacuoles at the same time that the spore initials are formed. There was no indication in any of the material studied that a sterile region was present on the site of the columella prior to the formation of the columella wall. This is a more primitive condition than that in *Mucor genevensis* and approaches the type seen in *Mortierella* where no columella is formed. No septum is formed between sporangiophore and columella (fig. 46).

The formation of the sporangiola presents several interesting features. The sporangiophore, instead of producing a single terminal swelling as in sporangium formation, develops instead numerous curiously constricted

dichotomous branches. Septa do not develop at the constrictions. The ultimate dichotomies develop terminal swellings, into which a few nuclei are carried by cytoplasmic streaming. These nuclei undergo several mitotic divisions and come to occupy a peripheral position in the swollen primary head. The center of this head becomes highly vacuolate, and is apparently entirely devoid of nuclei. Upon its surface numerous small swellings arise and enlarge rapidly to form the fundaments of the sporangiola.

The base of the sporangiolum fundament is very narrow and forms a short but distinct stalk. The nuclei of the primary head migrate through this basal stalk into the developing sporangiolum. As far as could be determined no nuclear division takes place within the sporangiolum, the entire nuclear complement of this structure being derived from the primary head. As the sporangiolum approaches its maximum development, longitudinally disposed vacuoles appear which soon separate the cytoplasm into several multinucleate segments (fig. 47). The number of segments is usually three, but as Thaxter (28) points out, a secondary cleavage may result in more than this number of spores being formed. Spore formation after this point differs in no appreciable way from that in the sporangium. As the spores are developing, the basal stalk of the sporangiolum loses its cytoplasmic contents and forms an almost spherical vesicle which functions as a sterigma (fig. 48).

As Thaxter and Weber and Wolf have indicated, all stages of intergradation between sporangia and sporangiola may be found in a single culture of this species. It is clear that these structures are quite homologous and that the sporangiolum represents an extreme modification of the sporangium. The homologies of the curious hyaline, deciduous vesicle formed from the basal stalk of the sporangiolum are not so easily interpreted, but it probably represents a highly modified prototype of the simple sterigma present in *Choanephora*.

The progametangial initials are multinucleate and occasional mitotic divisions are seen in them. As the progametangia are delimited from the intertwined hyphae at the point of contact of the oppositely sexed mycelia, they become broadly clavate or somewhat lobulate and their nuclei are increased by further mitosis. They become somewhat flattened where their tips are in contact (fig. 37) and, after a short period where both progametangia are enlarging rapidly, the wall between them is dissolved; at approximately the same time the suspensors are delimited from the zygosphoric hyphae and from the gametangia by transverse septa (fig. 38). As the wall which separates the gametangia is broken down, the contents of the two cells undergo plasmogamy and continued growth of the fusion cell proceeds. The nuclei in the young coenozygote divide mitotically several times, and there come to be approximately 10–20 nuclei in the cell. About 6 hours after the formation of the coenozygote most of the nuclei enlarge considerably and

approach each other, remaining for some hours associated in pairs (figs. 39, 40). As the exospore wall appears, about 15 hours after the formation of the coenozygote, these paired nuclei fuse. The fusion nuclei remain in the expanded phase and at this stage are the most conspicuous bodies in the young zygospore. Very shortly after fusion takes place the cytoplasm becomes vacuolate and oil plastids make their appearance and lie scattered at random in the cell (fig. 41). These oil plastids contain a bright orange oily substance which imparts the characteristic color to the zygospores. The fusion nuclei remain in the expanded state until the zygospore is at least 6 days old (fig. 42). During this period the oil plastids tend to group around the central vacuoles in the cell, so that when viewed externally the spore appears to contain a single oil globule.

Between the sixth and tenth day after the formation of the coenozygote the expanded fusion nuclei disappear and smaller apparently unfused nuclei become evident. These smaller nuclei are several times as numerous as the fusion nuclei. Although the actual divisions were not seen, it is thought probable that meiosis occurred at this point and that these smaller nuclei are haploid. At this point the abundance of oil plastids in the cell renders the interpretation of nuclear structures somewhat hazardous. Shortly after this point the haploid nuclei assume the unexpanded phase, the oil plastids accumulate densely in the cytoplasm and the zygospore enters the dormant condition (fig. 43). No further changes ensue until germination. The behavior of the mitochondrial elements during the formation and development of the coenozygote follows the course described for the preceding species, and show the same connection with the oil plastids.

Germination of the zygospores takes place in the third month after their formation, and is identical with that seen in the species of *Zygorhynchus* studied (fig. 44). The germ tube does not form a sporangium directly, but produces instead an extensive submerged vegetative mycelium which ultimately bears sporangiophores. Repeated attempts have been made to induce the germ tube to develop directly into a sporangiophore by isolating germinating zygospores upon non-nutrient and solid substrates, but all the germ tubes aborted and no further growth ensued. It seems possible that the limited amount of cytoplasm present in these small zygospores may prohibit the formation of a complex sporangiophore. Segregation of sex is apparently complete at meiosis, all the spores of any given sporangium giving rise to mycelium of only one sex.

DISCUSSION

A consideration of the nuclear behavior throughout the life cycle of the 8 species studied here indicates that except for minor differences in size and number of nuclei the same basic behavior-pattern is common to all of them. The salient features of this pattern are as follows: the haploid condition is

maintained throughout the development of the thallus; karyogamy occurs during the development of the coenozygote usually as the exospore appears, and all the nuclei present in the coenozygote fuse in pairs; the number of the nuclei in the coenozygote is not large, rarely exceeding 100; at the time of syngamy the nuclei enlarge considerably; no degeneration of unfused nuclei is evident; reduction division follows karyogamy closely and takes place before the zygote enters the dormant condition; the dormant period persists from 1–6 months, depending upon the species; at the time of germination either a single sporangiophore or an extensive submerged germ mycelium is produced by the zygospore; the spores produced in the germ sporangia are all of the same sex, indicating that sex segregation has been complete at the time of meiosis. This type of nuclear behavior in the zygote is correlated with a general similarity in the behavior of the nuclei during sporangium and sporangiospore formation. A progressive specialization can be noted in the amount of cytoplasmic segmentation in the sporangium, during spore formation, from the condition observed in certain cases in *Zygorhynchus* where no cytoplasmic segmentation occurs to that in *Absidia spinosa* where ultimately uninucleate portions of cytoplasm are cut out. In the zygospore a somewhat similar situation may be noted in the gradual reduction in the number of nuclei incorporated in the coenozygote from the condition in *Mucor genevensis* where many nuclei are present towards that in *Blakeslea trispora* where only a few nuclei enter the developing coenozygote. This reduction in nuclear number seems to be associated with an increase in nuclear size. It is possible that when further investigations have been carried out on intermediate forms valuable clues to the phylogenetic development of this group may be obtained from a comparison of nuclear behavior in different species. However, with the meagre evidence available at present any such phylogenetic speculation seems unwarranted.

The foregoing study has revealed several points which require further investigation before a satisfactory explanation can be offered. Sex segregation in some of the heterothallic forms, as outlined under the description of *Mucor hiemalis*, appears quite anomalous. It is probable that due to the small size of the nucleus and the obvious impossibility of tracing an individual nucleus through the life cycle, a satisfactory answer will only be obtained through genetical analysis of the progeny of many zygospore germinations. The relationship of the elements of the chondriome to the elaboration of the reserve substances in the zygospores, and other resistant structures and in particular the relation of the mitochondria to the developing oil plastids needs further study. The possible connection between the oil plastids and the syngamic nuclei must also be considered. Further investigations along these lines are contemplated. A subsequent paper in this series will outline the nuclear behavior in 6 additional species of the Mucorales.

SUMMARY

1. By the use of several techniques not previously applied to this group the nuclear behavior in 9 species of the Mucorales is traced through all the stages in the life history.

2. In these species, with the exception of minor differences in nuclear number and size, the same basic behavior is observed at all stages. This will be referred to as the "*Mucor* pattern" of nuclear behavior.

3. In the "*Mucor* pattern" all functional zygospore nuclei undergo karyogamy followed by immediate reduction division prior to the onset of dormancy in the zygospore. Segregation of sex is complete in the heterothallic species at meiosis.

4. In an azygous variety, *Zygorhynchus vuilleminii* var. *agamus*, no nuclear fusion or reduction is encountered in the azygospore which, in this case, cannot be regarded as a true sexual spore.

5. The relationship of the chondriome to the deposition of reserve substances is discussed and the possible connection between the mitochondria and the developing oil plastids is suggested.

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VITAMIN DEFICIENCIES OF TRICHOPHYTON DISCOIDES

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Mackinnon (8) reported that strains of *Actinomyces* stimulated the growth of *Trichophyton discoides* on a glucose-peptone agar. He found that the addition of thiamine to the agar medium had the same effect as *Actinomyces* on the growth of *Trichophyton*, and called attention to the considerable variation in the character of colony growth produced by the addition of thiamine to a glucose-peptone agar. From his results it appears that *T. discoides* suffers from a thiamine deficiency which is not satisfied by the thiamine in peptone[1] in the amount used, but is by thiamine synthesized by *Actinomyces albus*.

The present paper is a further report on the nutritional requirements of *Trichophyton discoides* Sabouraud, with special reference to its vitamin deficiencies. Part of the experimental work was performed at the Institute of Hygiene in Montevideo, Uruguay, and the balance at the New York Botanical Garden.

MATERIALS AND METHODS

The strain of *T. discoides* originally employed by Mackinnon was used in all experiments. Unless otherwise indicated the organism was grown at 35° C. on agar slopes in pyrex test tubes, 20 × 150 mm. Each tube contained 8 ml. of a basal medium composed per liter of 50 g. dextrose, 1.5 g. KH_2PO_4, 0.5 g. $MgSO_4 \cdot 7H_2O$, 2.0 g. asparagine, and 15 g. purified agar. To this medium the following trace elements in p.p.m. were added: 0.005 B, 0.02 Cu, 0.1 Fe, 0.01 Ga, 0.01 Mn, 0.01 Mo, and 0.09 Zn. The dextrose was Corn Products C.P. The asparagine was purified by treatment with Norit A and recrystallization from redistilled alcohol. The agar was purified by extraction with aqueous pyridine and alcohol (14). The stock cultures were maintained on the basal medium to which 10 mμ moles of thiamine and 100 mg. of neopeptone were added to each tube. Inoculations were made by transferring from a stock culture a bit of mycelium about the size of a pinhead.

In estimating the effects of various media on the growth of *Trichophyton* observations were made at intervals on the character of the growth and colony diameters were measured.

EXPERIMENTS

Relation to Thiamine and its Intermediates. *T. discoides* was found

[1] Robbins and Schmidt (12) found peptone to have a small amount of thiamine or its intermediates.

to require molecular thiamine. Its thiamine deficiency could not be satisfied by pyrimidine,[2] by thiazole or by a mixture of the two intermediates.

This was determined by testing the effect of thiamine and its intermediates on the growth of the organism on a dextrose-peptone medium. Although bacto-peptone contains little thiamine, the small amount present was destroyed by heating the peptone under alkaline conditions. A 33.3 per cent solution of peptone was heated at pH 10.0 for 20 minutes at 135° C. The reaction was then adjusted to pH 7.0. Tests with *Phytophthora cinnamomi* demonstrated that this treatment destroyed all, or very nearly all, the thiamine originally present in the peptone. A medium was prepared containing per liter 18 g. agar, 40 g. dextrose, and 30 ml. of the "heated peptone" solution. Fifty ml. of this medium or the same medium supplemented with thiamine or its intermediates were placed in flasks with a flat side 5 cm. by 14 cm. After sterilization the media were inoculated with *T. discoides*. The experiment was performed in triplicate and the results are given in table 1. Good growth was obtained only in those flasks to which thiamine was added; in fact pyrimidine at the amounts used appeared to be somewhat toxic.

TABLE 1. *Effect of thiamine and its intermediates on growth of Trichophyton discoides on a dextrose-agar medium containing peptone heated under alkaline conditions to destroy thiamine*

Additions to basal medium containing "heated peptone"	Development of Trichophyton
None	Scanty glabrous growth.
100 or 1000 mμ moles thiamine	Velvety colonies measuring 15 to 20 cm. in diameter after 15 days.
100 or 1000 mμ moles thiazole	Scanty glabrous growth.
100 or 1000 mμ moles pyrimidine	No growth.
100 mμ moles of thiazole and of pyrimidine or 1000 mμ moles each	No growth.

In a second experiment 20 ml. of the medium were used in Erlenmeyer flasks. Thiamine or its intermediates were added in amounts of 1, 5, and 20 mμ moles per flask. Abundant growth was obtained in those flasks containing thiamine; the development was somewhat greater with 5 and 20 mμ moles than with 1 mμ mole. Growth in the medium with no supplements was scanty; it was scanty also in the media containing the various amounts of thiazole, in those with 1 mμ mole or 5 mμ moles of pyrimidine, and in those with 1 or 5 mμ moles of both intermediates. No growth was observed in the flasks containing 20 mμ moles of pyrimidine, again indicating injury from the pyrimidine. Other experiments seemed to prove that the inhibitory effect of the pyrimidine was reduced or eliminated in media containing sufficient thiamine. Study of the apparent antagonism between pyrimidine and thiamine was not pursued further.

[2] The terms pyrimidine and thiazole are used in this paper to refer to 2-methyl-5-bromo-methyl-6-amino-pyrimidine and 4-methyl-5-β-hydroxyethyl-thiazole respectively.

Relation to Vitamins other than Thiamine. Although *T. discoides* grew quite satisfactorily on a mineral-dextrose-peptone agar supplemented with thiamine, it did not grow on the same medium with peptone replaced by minerals and asparagine. Evidently peptone supplied something essential for the growth of the organism not included in the minerals and asparagine by which it was replaced. Peptone is a complex mixture, containing minerals, amino acids, and several vitamins as well as other types of organic compounds. Since asparagine is a good source of nitrogen for most fungi and the basal medium contained all the minerals recognized as necessary, our attention was naturally directed toward the effect of vitamins other than thiamine.

A preliminary experiment showed that the addition to the basal medium of a mixture of eleven vitamins or vitamin-like substances allowed *T. discoides* to grow (table 2). The vitamins were added to each tube in the fol-

TABLE 2. *Effect of various supplements on growth of T. discoides on agar medium containing minerals, dextrose, asparagine and thiamine*
See text for composition of vitamin mixture and amino acids.

Additions to 8 ml. of basal agar medium containing 10 mμ moles thiamine	Average colony diameter in mm. after	
	13 days	18 days
None	0.0	0.0
Vitamin mixture	7.0	7.5
0.1 ml. D_R fraction	0.0	0.0
Vitamin mixture and 21 amino acids	7.0	7.5
Vitamin mixture, 21 amino acids and 0.1 ml. D_R fraction	10.0	13.0
Vitamin mixture and D_R fraction	10.0	13.0
Peptone 100 mg.	16.0	27.0

lowing amounts: pimelic acid 10 μg., para-aminobenzoic acid 10 μg., 2-methyl-1,4-naphtho-hydroquinone diacetate 5 μg., calcium pantothenate 5 μg., lactoflavin 10 mμ moles, nicotinamide 5 mμ moles, thiamine 5 mμ moles, pyridoxine 5 mμ moles, guanine 5 mμ moles, hypoxanthine 5 mμ moles, and *i*-inosotol 1 mg. The colonies produced in the presence of the vitamins were white, velvety, raised several mm., convoluted and with sharp well-defined edges. They were not so extensive as those which developed on a thiamine-peptone agar where the growth was flatter, less convoluted, and in our experiments somewhat gray brown in color.

Relation to Thiamine, Pyridoxine, and I-Inositol. To determine which of the eleven vitamins favorably affected the growth of *T. discoides* the organism was grown on the basal medium plus ten of the vitamins, omitting one after another of the eleven in turn. For example, one set of tubes contained all but pimelic acid, another all but para-aminobenzoic acid, a third all but calcium pantothenate and so on. It was found that no growth occurred when thiamine, pyridoxine, or *i*-inositol was omitted, even though the

other ten vitamins were present. Omission of any one of the other seven had little effect on the growth of the organism.

It appeared, therefore, that *T. discoides* suffered from a complete deficiency for thiamine, pyridoxine and *i*-inositol. This was confirmed by cultivating the organism on the basal medium supplemented per tube with 5 mμ moles thiamine, 5 mμ moles pyridoxine, and 1 mg. *i*-inositol singly and in all possible combinations (table 3). At the same time tubes were pre-

TABLE 3. *Effect of thiamine, pyridoxine, i-inositol and other supplements on growth of T. discoides on agar medium containing dextrose, minerals and asparagine*
See text for vitamin mixture.

Additions to 8 ml. of basal medium	Average colony diameter in mm. after		
	12 days	23 days	30 days
5 mμ moles thiamine	0.0	0.0	0.0
5 mμ moles pyridoxine	0.0	0.0	0.0
1 mg. *i*-inositol	0.0	0.0	0.0
thiamine and pyridoxine	0.0	0.0	0.0
thiamine and inositol	0.0	0.0	0.0
pyridoxine and inositol	4.5 (thin)	5.0 (thin)	5.0 (thin)
thiamine, pyridoxine and inositol	6.0	8.5	14.0
vitamin mixture	6.0	10.0	11.0
thiamine and 100 mg. peptone	22.0	30.0	31.0
thiamine, peptone and inositol	23.0	32.0	32.0
thiamine, pyridoxine and calcium phytate	4.0 (flat)	7.5 (flat)	9.0 (flat)

pared containing the basal medium plus the mixture of eleven vitamins in the amounts previously given and the basal medium plus 5 mμ moles of thiamine and 100 mg. peptone per tube. No growth was observed even after more than 30 days in the tubes supplemented with thiamine, with pyridoxine, with *i*-inositol, with thiamine and pyridoxine, or with thiamine and *i*-inositol. A thin, flat, waxy colony developed in the tubes containing the basal medium plus pyridoxine and inositol which increased little in size after the 12th day of incubation. Sub-cultures from these colonies made at the end of 34 days failed to grow on the basal medium supplemented with pyridoxine and *i*-inositol but grew normally on the medium supplemented with thiamine, pyridoxine, and *i*-inositol. We concluded that the small amount of growth on the medium supplemented with pyridoxine and *i*-inositol was the result of a minute amount of thiamine carried over with the inoculum.

Growth on the basal medium plus all three vitamins was quite satisfactory (fig. 1), and about the same as on the medium supplemented with the eleven vitamins. However, growth was much more rapid (table 3) on the basal medium to which thiamine and peptone were added. This experiment confirmed the conclusion that *T. discoides* has complete deficiencies for thiamine,

pyridoxine and *i*-inositol. Unless all three of these substances were present in the medium no growth occurred.

Since the fungus grew on the basal medium supplemented with thiamine and peptone, it follows that peptone in the amount used supplied sufficient pyridoxine and *i*-inositol or physiologically equivalent substances to satisfy the needs of the organism. However, the considerably more rapid growth on the peptone medium as compared with that on the medium supplemented

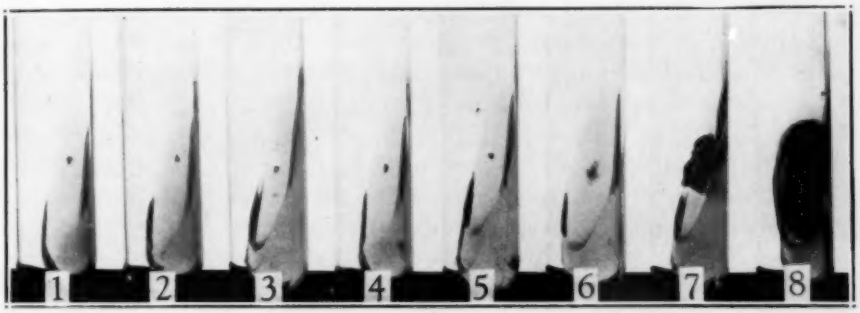

FIG. 1. Growth of *T. discoides* on a basal mineral-dextrose medium containing asparagine and 1.5 per cent purified agar supplemented with (1) thiamine; (2) pyridoxine; (3) *i*-inositol; (4) thiamine and pyridoxine; (5) thiamine and inositol; (6) pyridoxine and inositol; (7) all three vitamins; (8) thiamine and peptone. Age 32 days.

with the three (or eleven) vitamins suggested that peptone contains some unidentified factor or factors important in the development of this organism.

Attempts to Substitute other Vitamin Combinations for Thiamine, Pyridoxine, and I-Inositol. In the course of our investigation a considerable number of vitamins alone or in combination were used as supplements to the basal medium. Growth was obtained with those combinations only which included thiamine, pyridoxine, and *i*-inositol. In addition to the various vitamins tested singly or in combination with negative results and reported earlier in this paper, adding the following to the basal medium did not permit growth; biotin; thiamine and biotin; pyridoxine and biotin; thiamine, pyridoxine, and biotin; thiamine and hypoxanthine; thiamine and a D_R fraction;[3] thiamine, hypoxanthine and a D_R fraction; thiamine and calcium pantothenate; thiamine, biotin and calcium pantothenate; thiamine and nicotinamide; thiamine, nicotinamide, and biotin; thiamine, nicotinamide, calcium pantothenate, and biotin; para-aminobenzoic acid; calcium pantothenate and para-aminobenzoic acid; calcium pantothenate and para-aminobenzoic acid; calcium pantothenate, para-aminobenzoic acid, and nicotinamide; thiamine, calcium pantothenate, para-aminobenzoic acid,

[3] The D_R fraction is the filtrate from an extract of white potato tubers which has been treated with charcoal (13).

nicotinamide and biotin; thiamine and pimelic acid; thiamine and lactoflavin; thiamine and 2-methyl-1,4naphtho-hydroquinone diacetate; thiamine, lactoflavin, *i*-inositol, pimelic acid, and 2-methyl-1,4-naphtho-hydroquinone diacetate; thiamine, pimelic acid, para-aminobenzoic acid, 2-methyl-1,4-naptho-hydroquinone diacetate, calcium pantothenate, lactoflavin, nicotinamide, guanine, hypoxanthine, *i*-inositol, and a D_R fraction.

Relation to Calcium Phytate and an Inositol Phosphatide. Comparisons of growth on the basal medium supplemented with thiamine, pyridoxine, and *i*-inositol, and on the same medium with inositol replaced with an equal weight of calcium phytate or a phosphatide containing inositol[4] were made. To the medium in each tube 5 mμ moles of thiamine, 5 mμ moles of pyridoxine and 1 mg. of inositol, calcium phytate or the phosphatide were added. *Trichophyton* developed slowly on the medium containing calcium phytate; there was little growth during the first week. At the end of 30 days the colony diameter was 9.0 mm. as compared to 14.0 mm. on the medium containing

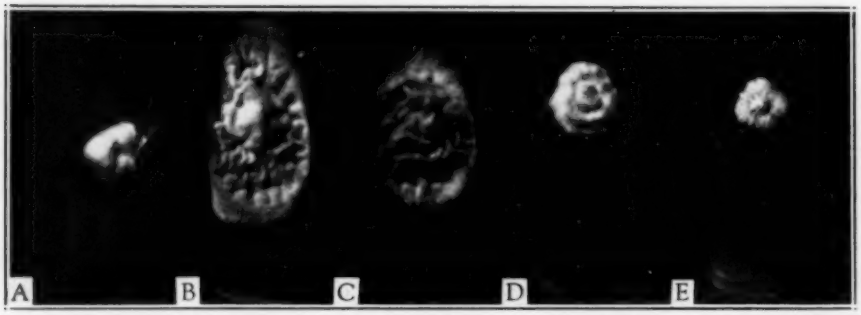

FIG. 2. *T. discoides* grown on a basal mineral-dextrose medium containing asparagine and 1.5 per cent purified agar supplemented with (A) thiamine, pyridoxine and inositol; (B) thiamine and peptone; (C) eleven vitamins and gelatin hydrolysate; (D) thiamine, pyridoxine and a phosphatide; (E) thiamine, pyridoxine and calcium phytate. Age 28 days.

inositol (table 3). The difference in growth was, however, greater than indicated by the colony diameters. On the phytate medium the colony was flat, closely appressed to the agar and with a powdery surface; on the inositol medium it was raised 3 or 4 mm. with a velvety surface. The phosphatide replaced inositol more nearly than did the calcium phytate. Colony diameters on the medium containing the phosphatide were equal to those on the inositol medium, but the colony height on the latter medium was 2 or 3 times that on the phosphatide medium (fig. 2). Neither calcium phytate nor the phosphatide was as satisfactory as inositol.

Effective Quantities of Thiamine, Pyridoxine, and I-Inositol. The effect of the quantity of each of the three vitamins for which *T. discoides*

[4] The phosphatide was kindly supplied by D. W. Wooley and is described in a paper published by Folch and Wooley (2).

shows a complete deficiency was determined by growing the organism on the basal agar medium containing a fixed and adequate amount of two of the vitamins with different quantities of the third. For inositol the medium contained per tube 10 mμ moles of thiamine and 50 mμ moles of pyridoxine with 2, 1, 0.5, 0.1, 0.01, and 0.00 inositol; for pyridoxine it contained 10 mμ moles of thiamine and 1 mg. of inositol with 50, 10, 1, 0.5, 0.01, 0.001, and 0.000 mμ moles pyridoxine; for thiamine we used 10 mμ moles of pyridoxine and 1 mg. of inositol with 10, 5, 1, 0.1, 0.01 and 0.000 mμ moles of thiamine.

At the end of two weeks no difference in the growth with from 2.0 to 0.1 mg. inositol was noticeable, but there was a marked reduction where 0.1 mg. was supplied. At the end of a month the growth with 0.5, 1, and 2 mg. inositol was about the same. There was a slight reduction with 0.1 mg. and a marked reduction with 0.01 mg. per tube; the colonies were flattened and grew closely appressed to the agar. There was little growth in the tubes lacking inositol, mostly under the agar and probably at the expense of inositol carried over with the inoculum (fig. 3).

In the tubes in which pyridoxine varied little difference in growth was noted at the end of two weeks with 50, 10, or 1 mμ mole of pyridoxine. There was a progressive reduction in growth in the tubes with 0.05 and 0.01 mμ mole and little difference between those with 0.001 mμ mole and the check. At the end of 4 weeks the growth in the tubes with 10 mμ moles and 1 mμ mole of pyridoxine was about the same, but in the former the growth was snow white, while in the latter it was somewhat grayish. With 0.05 mμ mole there was a distinct flattening of the colony, with 0.01 mμ mole the colony was reduced in diameter and quite flat, growth with 0.001 mμ mole was only slightly better than the check (fig. 3).

At the end of two weeks little difference was noted in the effects of 10, 5, and 1 mμ mole of thiamine, but with 0.1 and 0.01 mμ mole there was a progressive reduction in growth. At the end of a month there was no difference between the growth in tubes containing 10 and 5 mμ moles of thiamine, but the colonies appeared grayish with 1 mμ mole. With 0.1 mμ mole of thiamine the colonies were flattened with a raised spot in the center and with 0.01 mμ mole the colonies were thin, grayish and closely appressed to the agar. The check showed still less growth (fig. 3).

It appeared from these results that between 0.1 and 0.5 mg. of inositol and between 1 and 10 mμ moles of pyridoxine and thiamine per tube gave maximum growth of *T. discoides* for 4 weeks in our basal medium. These figures would be reduced for shorter periods of growth.

Inositol in Agar, Gelatin, and other Natural Products. The ability of *T. discoides* to grow on a thiamine-peptone agar demonstrated the presence in peptone of both pyridoxine and *i*-inositol or physiologically equivalent substances. One hundred mg. of neo-peptone per tube appeared to supply suf-

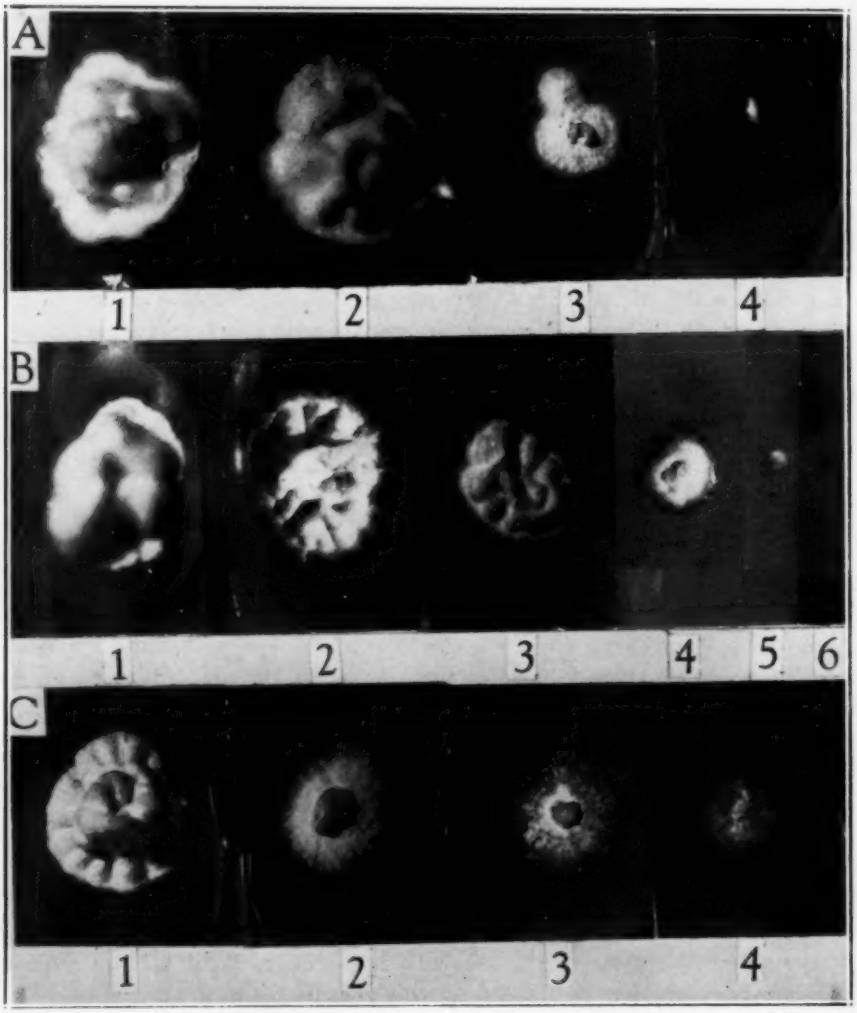

FIG. 3. Amount of vitamins and growth of *Trichophyton discoides* on mineral-dextrose medium containing agar and asparagine and supplemented as follows: A. 10mμ moles thiamine, 50 mμ moles pyridoxine and (1) 0.5 mg. *i*-inositol; (2) 0.1 mg. *i*-inositol; (3) 0.01 mg. *i*-inositol; (4) no inositol. B. 10 mμ moles thiamine, 1 mg. *i*-inositol and (1) 10 mμ moles pyridoxine; (2) 1 mμ mole pyridoxine; (3) 0.05 mμ mole pyridoxine; (4) 0.01 mμ mole pyridoxine; (5) 0.001 mμ mole pyridoxine; (6) no pyridoxine. C. 10 mμ moles pyridoxine, 1 mg. *i*-inositol and (1) 1 mμ mole thiamine; (2) 0.1 mμ mole thiamine; (3) 0.01 mμ mole thiamine; (4) no thiamine. Age 4 weeks. About twice natural size.

ficient *i*-inositol for maximum growth since the addition of inositol to the thiamine-peptone medium did not improve growth. One hundred and twenty-five mg. of Difco agar contained less *i*-inositol than required to induce growth of *T. discoides*. We failed to observe any growth on 8 ml. of our basal medium

prepared with unpurified Difco agar and supplemented with thiamine and pyridoxine. The D_R fraction from potato containing 13 mg. of dry matter furnished enough *i*-inositol to permit some growth though considerably less than the maximum.

I-inositol or physiologically equivalent material was found to be present also in hydrolyzed gelatin, hydrolyzed casein, and hydrolyzed egg albumen.[5] This was demonstrated by growing the fungus on the basal medium supplemented per tube with 10 mμ moles of thiamine to which 100 mg. of hydrolyzed gelatin, egg albumen, or casein were added. At the end of 3 weeks colonies with the following diameters had developed: on gelatin, 19 mm.; on the egg albumen, 18.3 mm.; on casein, 25 mm.; on peptone, 34.6 mm. The diameter on the basal medium plus thiamine, pyridoxine, and inositol was 8.3 mm. It is not possible to state the quantity of inositol present in these products from this observation but it was appreciable.

Relation to Unidentified Factors. Attention has been called above to the probability that peptone contains some unidentified factors important in the growth of *T. discoides*. This was suggested by the more rapid growth observed on a medium containing thiamine and peptone than on one containing thiamine, pyridoxine, and *i*-inositol. The unidentified factors were not found among the following substances: biotin, hypoxanthine, guanine, pimelic acid, para-aminobenzoic acid, calcium pantothenate, lactoflavin, nicotinamide, or 2-methyl-1,4-naphtho-hydroquinone diacetate. The addition of these vitamins to the basal medium containing thiamine, pyridoxine and *i*-inositol was of little or no benefit. We failed also to improve the basal medium containing the 12 vitamins listed above by the addition of 21 amino acids.[6] The further addition per tube of 100 mμ moles of choline, adenine, xanthine, cytosine, orotic acid, thymine, and uracil was not found to be beneficial. No benefit was observed by the addition per tube of 20 μg. of a concentrate of folic acid and of 23 amino acids[7] to the basal medium containing the 12 vitamins, 21 amino acids, and the pyrimidine and purine bases listed above.

[5] Difco gelatin was hydrolyzed by boiling for 24 hours with 8N H_2SO_4. The sulfuric acid was removed by treatment with $Ba(OH)_2$. Difco egg albumen coagulated was hydrolyzed in the same way. The hydrolyzed casein was a product from Mead Johnson and Co. with the laboratory number 92–403.

[6] These amino acids and the amounts added per tube were as follows: 0.15 mg. *d*-arginine, 0.075 mg. *dl*-α-alanine, 0.15 mg. *l*-aspartic acid, 0.15 mg. *d*-glutamic acid, 0.075 mg. glycine, 0.05 mg. β-alanine, 0.02 mg. *dl*-lycine HCl, 0.01 mg. *dl*-norleucine, 0.01 mg. serine, 0.01 mg. *dl*-threonine, 0.01 mg. *dl*-valine, 0.01 mg. *d*-cysteine HCl, 0.02 mg. *l*-histidine HCl, 0.01 mg. *l*-hydroxyproline, 0.01 mg. *dl*-methionine, 0.01 mg. *dl*-phenylalanine, 0.01 mg. *l*-proline, 0.02 mg. *l*-tryptophane, 0.005 mg. tyrosine and 0.005 mg. thyroxine.

[7] The amino acids included 0.1 mg. each of *dl*-α-amino butyric acid, *dl*-β-amino-*n* butyric acid, α-amino isobutyric acid, *dl*-α-amino caproic acid, *dl*-α-amino-*n*-valeric acid, *dl*-δ-amino-*n*-valeric acid, *dl*-aspartic acid, betaine HCl, *dl*-benzoylalanine, creatinine, *d*-cysteine, glutamine, glutathione, *dl*-homoaptine, *m*-nitro hyppuric acid, *p*-nitrophenylglycine, *n*-phenylglycine, pyrazine monocarboxylic acid, pyrazine 2, 3 dicarboxylic acid, sarcosine, spermadine phosphate, and taurine.

In spite of these negative results it may be entirely possible that the unidentified factors are included among the amino acids or other compounds tested. The active compound, if it was one of the 60 substances tested with negative results, may have been used in too small an amount, or its activity may have been masked by the toxicity of another member of the complex.

The unidentified factor or factors appeared to be present in a D_R fraction prepared from potato, in malt extract, in casein hydrolysate, in Difco gelatin both hydrolyzed and unhydrolyzed, and in egg albumen hydrolysate. The addition per tube of a D_R fraction containing 13 mg. of dry matter to the basal medium plus the 12 vitamins was beneficial though not as much so as the addition of an equal amount of neo-peptone. The benefit observed from 100 mg. of Difco desiccated malt extract was about equivalent to that obtained with 13 mg. of the D_R fraction. Difco gelatin and casein hydrolysate were almost as beneficial as peptone when compared on a dry weight basis. Some benefit was obtained with the addition per tube of 4 mg. of peptone or gelatin and perhaps with 1 mg.; 16 mg. doubled the growth as measured by colony diameters, and the effect increased still further with 64 or 128 mg. per tube (table 4). The action of the gelatin, casein, or peptone was not the

TABLE 4. *Effect of various additions to an agar medium containing dextrose, minerals, asparagine and eleven vitamins on the growth of T. discoides.*
The unit for the D_R fraction was 1.3 mg. instead of 1.0 mg.

Additions to 8 ml. of basal medium plus 11 vitamins	Average colony diameter in mm. after 14 days					Average colony diameter in mm. after 24 days				
	Gelatin	Peptone	Asparagine	Casein	D_R fraction	Gelatin	Peptone	Asparagine	Casein	D_R fraction
0	6.0	6.0	6.0	6.0	6.0	11.0	11.0	11.0	11.0	11.0
1 mg.	7.5	4.0	4.0	.....	7.5	14.0	11.0	10.0	.....	13.0
4 mg.	9.0	4.0	5.0	.....	.....	15.0	14.0	11.0	.....	.....
16 mg.	12.0	13.0	6.0	17.0	11.0	25.0	27.0	10.0	23.0	22.0
64 mg.	15.0	20.0	4.0	18.0	.....	29.0	32.0	8.0	32.0	.....
128 mg.	15.0	20.0	4.0	.....	6.0	35.0	34.0	8.0	.....	11.0

result of increasing the nitrogen supply. This followed because increasing the asparagine had no comparable effect. Since gelatin was beneficial the effect of 4 mg. of glycine per tube was tested. No benefit was observed. Further work on the nature of the unidentified factors is in progress.

DISCUSSION

T. discoides is parasitic on calves and produces an infection of man which presents the same clinical characteristics. *T. album* is very similar to *T. discoides* in its pathogenic effects and is distinguished from the latter organ-

ism by differences in the appearance of colonies upon Sabouraud's medium. However, *T. discoides* closely resembles *T. album* when grown on Sabouraud's dextrose medium to which thiamine had been added and an investigation of *T. album* demonstrated that it synthesized thiamine. This suggested that *T. discoides* may be a mutant of *T. album*, a mutation which involved the loss of the ability to synthesize thiamine. These observations emphasize also the importance of the vitamin content of a medium in determining the diagnostic characteristics of an organism which is identified largely by its colonies.

The nutritional requirements of species of *Trichophyton* have received relatively little attention. Mosher, Saunders, Kingery and Williams (9) found that *T. interdigitale* did not grow in a mineral-sugar medium containing amino acids. Addition of crystalline thiamine, *i*-inositol, a highly concentrated preparation of pantothenic acid, or crude lactoflavin permitted growth. Some combinations of these supplements were more effective than the single substances. Oyama (10) reported that thiamine had no effect on the growth of *T. crateriforme* in a solution of minerals, sugar, and asparagine, but a concentrate of rice polishings improved growth. On the other hand the growth of *T. rosaceum* was improved by the addition of thiamine or of a concentrate of rice polishings. Peck and Rosenfeld (11) state that the growth of *T. gypseum* was not affected by vitamins A, B, and D, but that from 0.09 to 0.5 per cent of vitamin C was injurious.

It is of interest to note that *T. interdigitale* and *T. discoides* both show deficiencies for thiamine and inositol. We found, however, no benefit from the addition of lactoflavin or pantothenic acid, both of which were beneficial to *T. interdigitale*. Mosher et al. did not determine the effect on *T. interdigitale* of pyridoxine, lack of which prevented growth of *T. discoides*. *T. interdigitale* differs from *T. discoides* also in ability to grow in the presence of any one of four vitamins. The latter organism required the presence of three vitamins in the medium, one or two of the three were not adequate. Mosher et al. (9) believe that *T. interdigitale* has "latent synthetic capabilities" and that "during a long growth period mechanisms are developed for the production of any one of a number of specific cell constituents which, under other conditions, may be taken from the environment as food." This did not seem to apply to thiamine, pyridoxine, and inositol for *T. discoides* as no growth occurred unless all three of the vitamins were present even when incubation was prolonged for more than 30 days.

Mosher et al. found various amino acids, especially leucine, hydroxy-α-amino butyric acid, aspartic acid, arginine, lysine, and phenylalanine, important in the nutrition of *T. interdigitale*. We obtained no benefit from the addition of numerous amino acids, including the six mentioned. On the other hand, the quantity of amino acids we used per culture was considerably

less than that used by Mosher et al. and a further investigation of the relation of *T. discoides* to amino acids is desirable. It is entirely possible that the unidentified favorable factors in peptone, casein, gelatin, and other natural substances are specific amino acids.

T. discoides is the only filamentous fungus known to the authors to have a complete inositol-deficiency. Eastcott (1) in 1928 found *i*-inositol to be a constituent of bios and important for the growth of a strain of yeast. Jansenns (4) reported inositol indispensable for the development of beer yeast. Williams and associates (15) found some strains of yeast to show a partial inositol-deficiency and others none. Some filamentous fungi have partial inositol-deficiencies (3, 6). Lash Miller, Kögl and Hasselt, and Wooley (7, 5, 18), found a high degree of specificity of inositol for yeast. Wooley (16, 17) reported inositol to be a growth factor for mice. Its deficiency resulted in stoppage of growth, paralysis of the hind quarters, and loss of hair. It seems clear that inositol is an important growth substance with a high degree of specificity, and further investigation will doubtless show deficiencies of various degrees in other organsms.

The complete inositol-deficiency shown by *T. discoides* suggests that this organism might be used for detecting and perhaps estimating inositol. However, before complete dependence can be placed on its use for the bio-assay of *i*-inositol further studies on specificity similar to those made on yeast should be made with this organism. The relatively slow growth of *T. discoides* and the need for an incubation temperature of 35° to 38° C. are disadvantages. Our own observations with this organism suggest that it may be useful in detecting the presence of inositol. For example, by bio-assay with *T. trichophyton*, it appears that agar contains little or no inositol while gelatin, casein hydrolysate, and albumen hydrolysate contain appreciable amounts.

SUMMARY

T. discoides suffers from complete deficiencies for pyridoxine, *i*-inositol, and molecular thiamine. In addition there are partial deficiencies for unidentified substances. The unidentified substances were present in peptone, casein hydrolysate, hydrolyzed egg albumen, malt extract, gelatin, and a filtrate (D_R fraction) prepared from white potatoes. No evidence was obtained that the partial deficiencies could be satisfied by biotin, lactoflavin, folic acid, pimelic acid, para-aminobenzoic acid, pantothenic acid, hypoxanthine, guanine, nicotinamide, 2-methyl-1,4-naphtho-quinone diacetate, seven pyrimidine and purine bases, and 43 amino acids. Ca phytate and a phosphatide containing inositol were less effective than *i*-inositol. The presence of appreciable quantities of inositol in gelatin, casein hydrolysate, and albumen hydrolysate was indicated by the growth of *T. discoides*. Unhydrolyzed agar appeared to contain little or no inositol. Maximum growth was obtained in a

4 weeks period in a medium containing between 0.1 and 0.5 mg. of inositol, and between 1 and 10 mμ moles of pyridoxine and thiamine.

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VARIATIONS IN ASCORBIC ACID AND DRY MATTER CONTENT OF COWPEA PLANTS AT DIFFERENT TIMES OF DAY

MARY ELIZABETH REID

The available data on variations in the ascorbic acid and dry matter content of cowpea plants at different times of day are presented here because of their possible bearing on the determination of a favorable time for harvesting fruits and vegetables and the special importance at this particular time of storing foods with the highest possible vitamin values. It has been recently pointed out by Auchter[1] that if we are to end with high nutritive values in the processing of fruits and vegetables, it is important that we start with them.

The observations herein reported were made during the growing seasons of 1939 and 1940 and were briefly mentioned in a previous publication (Reid 1942). It has been shown previously that losses of ascorbic acid occur at night (Reid 1939; Moldtmann 1939; Smith and Gillies 1940; Kohman and Porter 1940; Reid 1940, 1941). Moldtmann found fluctuations in the ascorbic acid content of leaves of deadnettles (*Lamium album*) with minimum values occurring at night and maximum values reached by mid-day or by 3 p.m. There was no significant difference in the vitamin C content of stems and petioles at different times. In fact, in these organs a small increase at night was found, but not always at the same time as the decrease in the leaves. With increasing light intensity and concomitant increase in assimilation synthesis of ascorbic acid was found to be favored, and to be depressed toward evening with the decreasing light intensity. No periodic variation was found in the ascorbic acid content of unripe fruits of elder (*Sambucus nigra*).

The literature dealing with the effect of seasonal and daily variations in light intensity upon ascorbic acid accumulation in plants has recently been reviewed together with the presentation of additional data obtained in a study with cowpea plants (Reid 1942). The interrelations of nitrogen and carbohydrates to ascorbic acid accumulation were discussed and it was suggested that weather conditions involving differences in light intensity should be considered in determining the time for harvesting fruits and vegetables. That a "time-of-day"[1] factor should also be considered with respect to vitamin C values, particularly of leafy types of vegetables is strongly suggested by the present data.

[1] In a lecture given April 1, 1942, on "Interrelations of soil, plant, and human nutrition."

PROCEDURE

The very young plants employed in some of the tests were grown in sand without added nutrients; the older plants used in other experiments were cultured in glass jars containing nutrient solutions. A large number of cultures were prepared in most of the tests so that it was possible to select plants uniform in size and appearance for the ascorbic acid and dry weight determinations. At the time of transfer from the dark chamber and before synthesis of carbohydrates had begun, ascorbic acid assays were made of some of the plants and dry weight determinations were made of others. Similar determinations were made with other plants at different times during the day with a final determination toward the end of the light period. Five plants were used in each assay with very young plants and the same number for dry weight determinations. Two plants were used in each determination with the larger plants. Duplicate or triplicate evaluations were made in most of the tests. The values per plant as presented represent averages of the different groups of plants used in each test. Microchemical tests were made on thin sections of stem and leaf tissues for starch (iodine reaction) and reducing substances (Flückiger reaction).

OBSERVATIONS

The seven-day-old plants tested on May 24th were exposed to light of high intensity throughout each daily light period from the time of their emergence above the sand. A similar group of plants, ten days old, was tested on June 5, when the light intensity was also high throughout the day though it had been low during the four preceding days. The results of these experiments are shown in table 1.

The plants tested on May 24th showed significant gains in total dry weight and in total ascorbic acid until some time between 11 a.m. and 2 p.m., maximum values having possibly been reached before 2 p.m. No significant increase in ascorbic acid per unit of fresh weight occurred after 11 a.m. These plants contained a large amount of starch and reducing substances, a consequence of the shortage of mineral nutrients and high daytime illumination throughout the development of the plants. The plants tested on June 5th, on the other hand, gained throughout the day both in dry weight and in absolute amount of ascorbic acid as well as in ascorbic acid content on a green weight basis. Because of the preceding cloudy weather the plants started the day with a much lower content of dry matter and ascorbic acid. It is evident that on June 5th, when the light conditions became favorable for photosynthesis, the plants had the capacity to synthesize and accumulate both dry matter and ascorbic acid throughout the course of the day. In both tests the increase in ascorbic acid was much greater on a green than on a dry weight basis. In fact, on May 24th, a loss on a dry

TABLE 1

Ascorbic acid and dry matter content of two groups of plants at different times of day and exposed to different light intensities preceding the day of the tests

Time of day	Dry wt. per plt. (mg.)	Per cent dry matter	C per plt. (mg.)	C in fresh wt. (mg./g.)	C in dry wt. (mg./g.)
May 24 Very bright, preceding days also bright (Plants 7 days old)					
8:00 a.m.	136	8.7	0.727	0.441	5.35
11:00 a.m.	148	9.2	0.793	0.498	5.35
2:00 p.m.	159	9.5	0.822	0.502	5.17
5:00 p.m.	162	9.5	0.824	0.497	5.09
8:00 p.m.	160	9.3	0.822	0.501	5.14
June 5 Very bright, four preceding days cloudy (Plants 10 days old)					
8:00 a.m.	167	7.5	0.664	0.290	3.98
11:00 a.m.	180	7.9	0.686	0.302	3.81
2:00 p.m.	193	8.4	0.755	0.335	3.91
5:00 p.m.	195	8.6	0.824	0.345	4.23
8:00 p.m.	206	8.7	0.871	0.368	4.23

weight basis is indicated during the course of the day, a consequence of a more rapid increase in dry weight than in ascorbic acid.

The results of one of the tests with older plants are shown in table 2. The plants were grown in a partially shaded greenhouse and were placed in full sunlight in the open during the day preceding the test as well as on the day of the test. On the day of the transfer (June 12) the illumination was high until 1 p.m., after which it was cloudy for the remainder of the day. On June 13th, the day of the test, the light intensity was high until toward sunset. Gains in both dry matter and ascorbic acid occurred throughout the day. Because of having been grown in partial shade and with an abundance of mineral nutrients the plants had not previously accumulated much reserve carbohydrates and their ascorbic acid content was relatively low. In previous studies it had been found that the leaves of cowpea plants of this age grown under high illumination tend to have approximately 1 milligram of ascorbic acid per gram of fresh tissue; whereas, the leaves of the present plants contained only 0.66 milligrams of vitamin C per gram at the beginning of the light period and 0.80 milligrams at the end of the day. The stems gained in dry weight throughout the day but no appreciable increase in ascorbic acid was indicated after 11 a.m. The results suggest that the roots increased in total ascorbic acid and in total dry weight until 2 p.m. As in the previous tests the changes in ascorbic acid during the course of the day were much less on a dry than on a green weight basis. The 11

TABLE 2

Variations in green and dry weights and in ascorbic acid content of cowpea plants at different times of day

(June 13) Time of day	Green wt. per plant (g.)	Dry wt. per plant (g.)	Percentage of dry matter	Ascorbic acid		
				Total per plt. (mg.)	Mg./g. (green wt.)	Mg./g. (dry wt.)
Leaves						
8:00 a.m.	9.08	0.98	10.8	5.99	0.660	6.11
11:00 a.m.	9.22	1.06	11.5	6.66	0.722	6.28
2:00 p.m.	9.30	1.14	12.2	7.09	0.762	6.22
5:00 p.m.	9.60	1.27	13.2	7.72	0.804	6.08
Stems						
8:00 a.m.	7.44	0.63	8.5	0.84	0.113	1.33
11:00 a.m.	7.22	0.67	9.3	1.19	0.165	1.78
2:00 p.m.	7.11	0.66	9.6	1.22	0.170	1.85
5:00 p.m.	7.54	0.74	9.8	1.12	0.148	1.51
Roots						
8:00 a.m.	6.31	0.30	4.8	1.45	0.230	4.83
11:00 a.m.	6.44	0.31	4.8	1.65	0.256	5.32
2:00 p.m.	6.97	0.36	5.2	1.78	0.255	4.94
5:00 p.m.	7.21	0.36	5.0	1.70	0.236	4.72
Entire plant						
8:00 a.m.	22.8	1.92	8.4	8.27	0.362	4.31
11:00 a.m.	22.9	2.04	8.9	9.50	0.415	4.65
2:00 p.m.	23.4	2.16	9.2	10.09	0.431	4.67
5:00 p.m.	24.4	2.37	9.7	10.54	0.432	4.45

a.m. ascorbic acid values are higher than those at 8 a.m., but there were no consistent gains on a dry weight basis after this time.

Another set of slightly younger plants was tested on July 10th. This set also had been grown in a partially shaded greenhouse and was placed in the open in full sunlight two days previous to assaying. On July 10th the illumination was high until 3 p.m., after which it became cloudy (average radiation about 1800 ft.-c.). Because of having been exposed to full sunlight for a longer period than the plants tested on June 13th, these plants contained more starch and sugars and also had the same absolute amount of ascorbic acid but an approximately 20 per cent higher content on a green weight basis at the beginning of the light period, although the plants weighed approximately 20 per cent less than the June 13th plants. The total ascorbic acid increased from 8.17 milligrams at 5 a.m. to 10.20 milligrams at 3 p.m., a daylight increase of approximately 25 per cent. There was no increase in total dry weight and an apparent loss in ascorbic acid after 3 p.m. when it became cloudy (see table 3).

TABLE 3

Variations in green and dry weights and in ascorbic acid content of plants at different times of day

(July 10) Time of day	Green wt. (g.)	Dry wt. (g.)	Percentage of dry matter	Ascorbic acid		
				Total per plt. (mg.)	Mg./g. (green wt.)	Mg./g. (dry wt.)
5:00 a.m.	18.83	1.68	8.9	8.17	0.434	4.86
9:00 a.m.	19.21	1.77	9.2	8.63	0.449	4.88
12:00 m.	18.95	1.93	10.2	9.51	0.502	4.92
3:00 p.m. (Cloudy after 3:00)	18.80	1.97	10.5	10.20	0.542	5.18
6:00 p.m.	19.89	1.97	9.9	9.60	0.482	4.87

DISCUSSION

A definite influence of a light intensity factor on a "day-to-day" and even on a "time-of-day" effect upon the ascorbic content of cowpea plants is indicated by these results. In all of the tests conducted thus far on this problem, increases both in total amount of ascorbic acid per plant and in the ascorbic acid content per unit of fresh weight occurred upon exposure of the plants to light. In some tests, increases tended to occur throughout the day, whereas in others they ceased relatively early. In previous tests with plants used in other experiments it was found that the ascorbic acid values of plants grown in partial shade were relatively low even at the end of the day.

It is well accepted that carbohydrate synthesis is influenced directly by light intensity and indirectly by the supply of stored carbohydrates and availability of mineral nutrients, particularly of nitrogen. The concomitant increases in ascorbic acid and dry matter during the course of the day and from day to day suggest a close relation between the synthesis of carbohydrates and that of ascorbic acid. There is need for further work to determine their quantitative relations by more exact methods and the interacting influence of nitrogen supply.

SUMMARY

Progressive increases in both ascorbic acid and dry matter in cowpea plants are shown to occur from the beginning of the daily light period, and to continue during the course of the day depending upon the carbohydrate-synthesizing capacity of the tissues.

Plants grown under high illumination and much-limited nutrient supply tend to have relatively large reserves of starch and sugars and a low carbohydrate-synthesizing capacity. Under favorable light conditions such plants tend to attain their maximum vitamin C values early in the day.

Plants subjected to prolonged periods of cloudy weather, and especially if an abundant supply of mineral nutrients has been available, tend to have low vitamin C values and a low supply of starch and sugars.

The results suggest that for best vitamin C values, harvesting of vegetables should be done not before mid-forenoon after generally clear weather; or if it must be done following cloudy days, collection should be made as late in the day as possible.

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BULLETIN OF THE TORREY BOTANICAL CLUB
69(7): 528–538 OCTOBER, 1942

PHYCOLOGICAL NOTES—I

GEORGE J. HOLLENBERG

HAPALOSPONGIDION was established by Saunders (1899) on material collected at Pacific Grove, California. It is a brown alga which forms cushion-like small gelatinous thalli on rocks in the upper littoral zone. As described by Saunders, it is composed of a basal distromatic layer bearing erect closely packed and mostly unbranched filaments, which are free except at the tips, and which adhere on account of copious quantities of mucilaginous material. Saunders described both unilocular and plurilocular sporangia. The former he described as terminal and usually single, although he also described and figured series of enlarged cells in the erect filaments, which he took for immature or abortive unilocular sporangia. Largely on the strength of the latter observation, it seems, Setchell and Gardner (1924, 1925) doubted Saunders' description of single terminal unilocular sporangia and concluded that *Hapalospongidion gelatinosum* Saunders should be united with *Microspongium* under the name *M. saundersii* S. & G. In this disposition of Saunders' plant these investigators seem to have disregarded the fact that in the type species, *Microspongium gelatinosum*, described by Reinke (1888), both types of reproductive structures are laterally inserted on the erect filaments.

Saunders' plant (fig. 14) has been found by the writer at a number of places along the coast of southern California and near Carmel in central California. It has also been found by the writer as far south as Punta Banda, Lower California, Mexico. Examination of no. 583 of the Hancock Expedition of 1934, collected at Petillan Bay, Mexico, by Dr. W. R. Taylor, shows that it is the same as Saunders' plant.

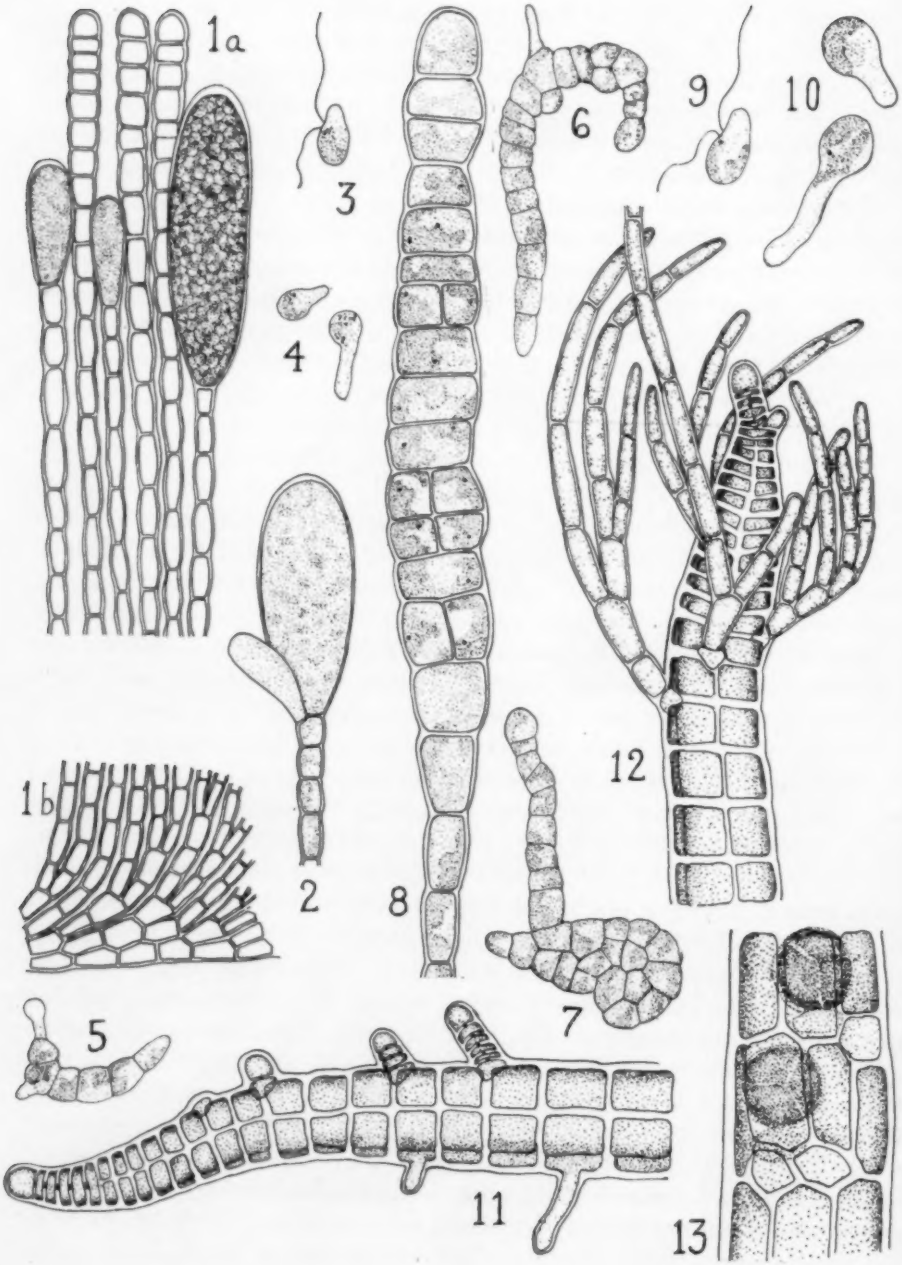
Near Corona del Mar in Orange County, California, the plant is usually abundant throughout the winter over an area several feet in diameter on a certain large rocky point. It has been observed at this locality for several successive seasons, and although it usually partially disappears during the winter months, it can be found at all seasons of the year. Both types of reproductive structures have been found a number of times. They correspond closely to Saunders' description and figures. Mature unilocular sporangia (fig. 1a) are relatively large, measuring 25–35 × 80–140 μ. They are oblong in shape and strictly terminal. They occur singly at the tips of the erect filaments. The tips of the sporangia are well below the tips of adjacent sterile filaments. No seriate intercalary unilocular sporangia were observed, and it seems probable that structures of such a nature reported by Saunders were abortive plurilocular sporangia. A few lateral outgrowths (fig. 2) were observed at the base of unilocular sporangia. These may be interpreted as

abortive accessory unilocular sporangia or possibly the true tip of the fruiting filament. In the latter case the unilocular sporangium could be thought of as actually lateral in position. A single very large nucleus is present in very young unilocular sporangia, suggesting that meiosis occurs in these reproductive structures as might well be expected, but no cytological studies were undertaken to determine the nature of the first nuclear divisions.

Normal plurilocular sporangia (fig. 8) occur on plants distinct from those which bear the unilocular sporangia, although both types of plants are often indistinguishably associated as a result of coalescence. The plurilocular sporangia are intercalary near the tips of the erect filaments, as described by Saunders. Their development is initiated by transverse divisions of cells immediately below the apical cell of a filament. One and usually two longitudinal divisions follow, resulting in the production of two tiers of four cells from each cell of the erect filament. These observations seem to necessitate retention of Saunders' genus *Hapalospongidion,* with *H. gelatinosum* as the type species.

Saunders does not describe motile reproductive cells of *Hapalospongidion* and did not study the development of germlings. The writer finds that zoids from both types of reproductive structures are of the usual form (figs. 3, 9), containing a single chromatophore and distinct stigma as well as two unequal flagella laterally inserted. Neither type of zoid gave evidence of fusing in the cultures. They were allowed to settle on cover-glasses and germling stages were studied in a manner similar to that employed for *Hapterophycus* (Hollenberg 1941). Examination of the cultures 12 hours after liberation of the zoids showed the formation of germ tubes (fig. 4). A single stigma was present at this time in germlings from both types of reproductive structures. It seems probable, therefore, that both types of zoids ordinarily germinate without fusing under culture conditions. The development of germlings from plurilocular sporangia was followed in the cultures for a period of one month. The entire contents of the germling initial usually migrates into the germ tube and is cut off from the old wall of the germling initial by a cross wall (fig. 5). Subsequent growth and cell divisions result in the formation of a short filament, usually provided with a single rhizoid. Longitudinal and irregular divisions, commonly accompanied by development of a sharp bend in the filament at the point of active cell division, soon initiate the development of a discoid thallus (figs. 6, 7). Although the development of the germlings was not followed beyond the development of this initial discoid thallus, it seems evident that the life cycle involves a facultative alternation of similar but distinct generations, parthenogenetic development of gametes seemingly repeating the haploid generation.

The writer has for some time been impressed with the similarity of the descriptions of certain other members of the Ralfsiaceae to *Hapalospon-*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gidion. *Mesospora* was described as a new genus by Madame Weber-van Bosse (1910), with *M. schmidtii* from the Malay archipelago as the type species. This plant was also found by the same investigator at several stations during the Siboga Expedition (Weber-van Bosse 1913). Later *Mesospora van-bossae* was described by Boergesen (1924) from Easter Island in the south Pacific. Still more recently Feldmann (1937) described *Mesospora mediterranea* from the Mediterranean. The writer has not been privileged to examine specimens of any of these species, but all are strikingly similar to *Hapalospongidion gelatinosum* in a number of important respects. In all of these plants the erect filaments are free except at the tips, where they are described as being united by a surface layer of the very gelatinous material surrounding the filaments. In all of these plants the plurilocular sporangia are intercalary, and separated from the tips of the filaments by one or more sterile cells, each cell in the fertile region dividing into the ultimate fruiting cells in a manner similar to that described by Saunders for the California plant. In all three plants the erect filaments arise from a basal stratum mostly two cells thick. In *M. mediterranea* Feldmann describes cell rows developing to some extent downward from this distromatic layer, and the writer has observed the same condition in *Hapalospongidion gelatinosum* in one instance. Unilocular sporangia are described as arising laterally at the base of erect filaments in the case of *M. schmidtii*, and Feldmann describes and figures lateral unilocular sporangia in the Mediterranean plant. These reproductive structures are not known in *M. van-bossae*. In *M. schmidtii* a series of large intercalary cells are described similar to those which Saunders interpreted as immature or abortive unilocular sporangia, but which as noted above are more probably abortive plurilocular sporangia.

The only important distinction between *Mesospora* and *Hapalospongidion* is the terminal position of the unilocular sporangia in the latter plant. However, as noted above, occasional unilocular sporangia bear lateral proliferations at the base, suggesting that in *Hapalospongidion* these reproductive

Explanation of figures 1–13

FIGS. 1–10. *Hapalospongidion gelatinosum* Saunders. FIG. 1a. Unilocular sporangia and paraphyses. × 500. FIG. 1b. Erect filaments of the thallus arising from the distromatic basal layer. × 500. FIG. 2. Immature unilocular sporangium showing rudimentary tip of filament (?). × 500. FIG. 3. Zoid from unilocular sporangium. × 1000. FIG. 4. Germinating zoids from unilocular sporangium. × 500. FIGS. 5–6. Germlings developing from zoids from plurilocular sporangia, one month old. × 500. FIG. 7. Germling from zoid from plurilocular sporangium, two months old. × 500. FIG. 8. Plurilocular sporangium at upper end of erect filament of thallus. × 1000. FIG. 9. Zoid from plurilocular sporangium. × 1000. FIG. 10. Germinating zoids from plurilocular sporangia. × 1000. FIGS. 11–13. *Lophosiphonia villum* (J. Ag.) S. & G. FIG. 11. Tip of prostrate branch showing rhizoids and adventitiously endogenous erect branches arising from the central cells. The latter are not shown. × 250. FIG. 12. Tip of erect branch showing arrangement of trichoblasts. × 250. FIG. 13. Portion of erect branch with tetrasporangia and double scar-cells. × 500.

structures may in some cases be thought of as essentially lateral. For the present it seems best, however, to retain *Mesospora* as a distinct genus.

A study of the type material of *Ralfsia pangoensis*, described by Setchell (1924) from Pagopago Harbor, Samoa, shows that this plant is a species of *Hapalospongidion*, with all the characteristic features of the genus, except that plurilocular sporangia are unknown. **Hapalospongidion pangoensis** (Setchell) Hollenberg, comb. nov., differs from *H. gelatinosum* chiefly in the shorter erect filaments, which are mostly under 200 μ long. The cells of the erect filaments are considerably shorter and more nearly cylindrical than in *H. gelatinosum*.

Pterochondria Hollenberg, gen. nov. Thallus polysiphonous, complanate, ecorticate, erect from a rhizoidal base embedded in the cryptostomata of *Cystoseira;* branching alternate, distichous, the branches slightly

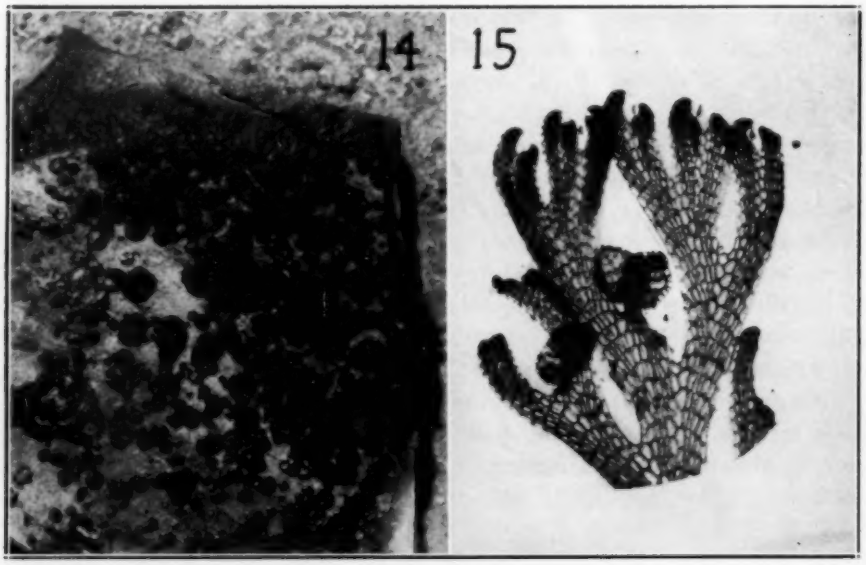

FIG. 14. Photograph of numerous plants of *Hapalospongidion gelatinosum* Saunders growing on a rock fragment. × 1. FIG. 15. Photomicrograph of a portion of *Pterochondria pygmaea* (Setch.) Hollenberg. × 30.

decurrent and of several to many orders, with mostly 3–5 segments between successive branches and with ultimate branchlets determinate; trichoblasts wanting; tetrasporangia one per segment, in straight series embedded in the adaxial side of the ultimate branchlets, tripartitely divided; antheridia covering the flat faces of flattened ovoid discs except for a sterile row of marginal cells; cystocarps globular ovoid, nearly sessile on the branches.

Thallus polysiphonus complanatus, ecorticatus, erectus ex fundamento radicato in cryptostomatibus *Cystoseirae* immerso; cum ramis alternantibus, distichis, aliquantum decurrentibus, ordinibus paucis aut multis, plerumque cum 3–5 segmentis inter ramos succedentes et cum ramulis ultimis determi-

natis; sine trichoblastis; tetrasporangiis singulis in segmentis in serie recta in latere adaxiale ramulorum ultimorum immersis, tripartite divisis; antheridiis superficies planas discorum complanatorum et ovatorum praeter ordinem sterilem cellulorum marginalium tegentibus; cystocarpiis ovoido-globosis fere sessilibus in ramis.

There are two known species of *Pterochondria:* **P. woodii** (Harv.) Hollenberg, comb. nov., is the type species; *Pterosiphonia woodii* (Harv.) Falkenberg, Rhodomelaceen, 274. 1901; Setchell and Gardner, Algae Northwest. Am., 329. 1903; *Polysiphonia woodii* Harvey, Ner. Bor. Amer. 52. 1853. This species is commonly 15–20 cm. high and occurs commonly on *Cystoseira* from the vicinity of Monterey, California, to British Columbia. It seems to occur frequently on *Pleurophycus* stipes also.

Pterochondria pygmaea (Setchell) Hollenberg, comb. nov.; *Pterosiphonia pygmaea* (Setchell) Kylin, Calif. Rhodophyceen, 38. 1941; *Pterosiphonia woodii* f. *pygmaea* Setchell, Phycotheca Bor.-Am. no. *1744.* 1911. This is a diminutive species mostly under 2 cm. high, known only from southern California, where it occurs chiefly on *Cystoseira osmundacea* (Menz.) Ag., but is also reported as occurring on *Pelagophycus porra* (Leman) Setchell.

Two features distinguish this genus from all known species of *Pterosiphonia:* the discoid antheridial branches (fig. 15), and the lack of a cylindrical creeping base. A very similar male reproductive structure occurs in *Chondria* and also in *Acanthophora* (cf. Boergesen 1918). Falkenberg (1901) states that the antheridial branches of *Pterosiphonia dendroidea* (Mont.) Falk. are cylindrical structures similar to those of *Polysiphonia.* The writer has observed male reproductive structures in several species of *Pterosiphonia* along the California coast. In all these species the antheridial branches seem to be cylindrical. It seems probable that this is true of all true species of *Pterosiphonia.* Falkenberg (1901) figures discoid antheridial branches for *Polysiphonia virgata* but it seems improbable that this will prove to be an accurate observation concerning a species of *Polysiphonia.*

Concerning the attachment organs of *Pterochondria,* F. S. Collins, in an unpublished manuscript on the Rhodomelaceae of North America, states that the spores germinate in the cryptostomata of *Cystoseira osmundaceae,* and that the young filaments are at first monosiphonous, but become polysiphonous soon after emerging from the cryptostomata.

The bluntly uncinate tips of determinate branchlets, especially evident in the case of young ramuli, seem characteristic of *Pterochondria.* This condition is seemingly due to a much retarded development of pericentral cells on the inner side of the branchlets. This also seems to account for the beak-like appearance of apices of branches, and possibly also for the staggered arrangement of the pericentral cells of a branchlet with respect to those of

the axis in the region where the branchlet is decurrent on the axis. Harvey (1853) mentions the latter condition indirectly in his original description of *Polysiphonia woodii*, when he speaks of the pericentral cells as being arranged in two axes.

TAENIOMA CLEVELANDII Farlow. *Taenioma perpusillum*, the type species, was originally described by J. G. Agardh (1851–1863) from material collected on the Pacific Coast of Mexico by Liebmann. The plant has since been collected from a number of places including points along the Atlantic coast of North America and in Europe. There was a difference of opinion whether the plant should be placed in the Delesseriaceae or the Rhodomelaceae until Thompson (1910) described the morphology and sexual reproductive structures and showed that the plant is a member of the Rhodomelaceae. *Taenioma clevelandii* was described by Farlow (1877) from material collected by Cleveland at San Diego, California. As pointed out by Farlow, the California plant is much larger, up to 10 cm. high, and lacks the fasciculate branching of *T. perpusillum*. However, De Toni (1924) makes *T. clevelandii* a synonym of *T. perpusillum*, without giving reasons for so doing. In the herbarium of the University of California are several specimens of *Taenioma* collected along the coast of California from San Diego to Central California. During the summer of 1941 the writer collected this relatively rare plant afloat near Pacific Grove, California. Examination of this material, which was tetrasporic, and the other specimens in the herbarium of the University of California, one or more of which bore tetrasporangia, but none of which bore sexual reproductive structures, has led the writer to conclude that *T. clevelandii* is amply distinct from *T. perpusillum*. It differs not only in size and lack of fasciculate branching, but in several more distinctive respects. In the first place many segments usually intervene between successive branches in *T. clevelandii*, whereas relatively few segments occur between successive branches in *T. perpusillum*. Furthermore, the branches do not end in hairs in any of the specimens of *T. clevelandii* examined by the writer. Farlow failed to mention two additional points of difference. *T. clevelandii* has a rhizome as *T. perpusillum* has, but the flanking cells, which in the genus *Taenioma* are marginal in position on the flattened branches, and which are only half as long as the pericentral cells, occur on all branches, including the basal rhizome, in *T. clevelandii*, rather than on the ultimate branches only as in *T. perpusillum*. Finally, rhizoids are all unicellular in the specimens of *T. perpusillum* available for examination, whereas those of *T. clevelandii* are usually composed of two cells. In *T. clevelandii* branches arise from the center of the flat faces of the axes, presumably from the central cell.

GELIDIUM CALOGLOSSOIDES Howe. This species was originally described by Howe (1914) from Peru, and seems not to have been reported in the

literature since. During the past several years the writer has repeatedly encountered a diminutive creeping species of *Gelidium* along the coast of southern California and several times also in the Monterey region in the central part of the state. Examination of type material shows that these plants must be placed with Howe's species, from which they differ only in the lack of dwarf ventral branchlets near the hapterous attachment organs, which Howe described as frequently present in the Peruvian plant, and which the writer observed in the type material. Examination of additional material from Peru will be necessary to determine whether or not this is a constant difference. Tetrasporangia occur in diagonal rows in the lateral and finally somewhat erect branchlets as in the Peruvian plant. The internal structure is also very similar.

LOPHOSIPHONIA VILLUM (J. Ag.) S. & G., figs. 11–13. *Lophosiphonia villum* (J. Ag.) Setchell and Gardner, Algae Northwest. Am. *329*. 1903; Kylin, Lunds Univ. Årsskr. N. F. Avd. 2. **37**: 40, 1941. *Polysiphonia villum* J. Agardh, Sp. Alg. II. 941, 1863. Plants erect, 5–10–(18) mm. high, from prostrate creeping branches 40–60–(80) μ diam., of segments 1–1.5 diam. long, without trichoblasts, attached by frequent unicellular rhizoids with expanded lobed tips; rhizoids arising as outgrowths from the middle of the pericentral cells from which they are not cut off by a cross wall; erect branches simple or sparingly branched 40–80–(100) μ diam., of segments 1–2 diam. long, arising at irregular intervals mostly every 2–4 segments in a strictly endogenous manner, at first arching toward the tips of the prostrate branches; lateral branches arising from erect branches exogenously or endogenously, independent of the trichoblasts which they may replace; pericentral cells 4, totally without cortication; chromatophores forming transverse bands in the pericentral cells; trichoblasts mostly infrequent and often wanting, but sometimes relatively abundant, irregular in occurrence but tending to occur one per segment in a left hand spiral with one fourth divergence when abundant, once or twice or sometimes thrice forked, rather stiff, often recurved or even coiled, 15–20 μ diam., 250–480 μ long, soon deciduous, leaving persistent scar-cells which sometimes divide to form double scar-cells (fig. 13); tetrasporangia 50–60 μ diam. one per short swollen segment in more or less continuous straight series; antheridial branches cylindrical, incurved, arising from the entire branch primordium, 70–170 × 20–35 μ, on short one-celled pedicels, without sterile tips; cystocarps ovoid, sessile, more or less erect, 150–190 μ diam.; plants dark reddish brown, attached in small tufts or forming a continuous stratum on rocks and other algae, often intermingled with other mat forming algae, frequent mostly in the middle and upper littoral zone, central California to tropical America.

This plant has been collected by the writer about 20 different times, mostly along the coast of southern California. In general the plants agree well with the original description by J. Agardh (1863). Professor W. A. Setchell has kindly allowed the writer to examine some unpublished notes

which he made while examining types in European herbaria. Among these is a note to the effect that no trichoblasts are present in the type material of *Polysiphonia villum* in the Agardhian herbarium. However, the writer finds that the presence of trichoblasts is a variable condition in this plant, as is also the degree of branching of erect branches. The plant is readily recognized locally by its diminutive size, and by the relatively little branched or unbranched erect branches arising endogenously from prominent and permanently prostrate branches. These characters seem to warrant placing the plant in *Lophosiphonia* as Setchell and Gardner (1903) and Kylin (1941) have done. However, in this plant, as in certain other species of *Lophosiphonia* and *Polysiphonia*, the vagueness of the characters distinguishing these two genera is most vexingly evident.

According to Falkenberg (1901) the most characteristic features of *Lophosiphonia* are: (1) relatively limited and mostly simple or sparingly branched erect branches from distinctly and permanently prostrate branches; and (2) adventitiously endogenous origin of all branches. Examination of several specimens of *Polysiphonia hemisphaerica* Aresch. from the Baltic Sea indicates that in this plant all branches arise endogenously and adventitiously, but the erect branches are several inches high and repeatedly branched. Further study may necessitate removal of this plant from the genus *Polysiphonia*, but it certainly cannot be placed in the genus *Lophosiphonia*. In *Lophosiphonia villum* all erect branches and most of the lateral branches to which they give rise are produced in an adventitiously endogenous manner. However, careful examination shows that lateral branches frequently arise exogenously by division of subapical cells. A further complicating feature is the fact that in *Lophosiphonia villum* the rhizoids arise from the prostrate branches as outgrowths of the pericentral cells from which they are not cut off by a cross wall. In this respect as well as the origin of antheridial branches from the entire trichoblast primordia, *L. villum* is closely similar to a species of *Polysiphonia* common along the Pacific Coast of North America, which has been commonly identified as *P. urceolata* (Lightf.) Grev., but is seemingly a distinct species. Furthermore, it should be noted in this connection that in a number of local species of *Polysiphonia* erect branches frequently arise endogenously from distinctly prostrate branches.

The foregoing considerations make it seem necessary to conclude that, if the genus *Lophosiphonia* is to be retained at all, its characteristic features need to be more clearly delimited if possible. Accordingly it is proposed that the concept of the genus expressed by Falkenberg (1901) be modified slightly so as to include only those Rhodomelaceous plants with a single tetrasporangium per segment, which have relatively prominent and strictly prostrate branches giving rise to erect branches in a strictly adventitious and

endogenous manner, and in which the erect branches are relatively short and simple or bear relatively few lateral branches in an adventitious and *mostly* in an endogenous manner. This characterization differs from Falkenberg's concept only in that it provides that some of the lateral branches may arise exogenously from the erect branches. It is admittedly relative, perhaps even more so than Falkenberg's concept of the genus, but this modification seems necessary if the genus is to be retained at all. There will probably continue to be some confusion concerning the limitations of the genus, but if one should adhere strictly to Falkenberg's concept of the genus, *Lophosiphonia villum* would need to be returned to the genus *Polysiphonia,* in spite of its habit, since not all branches arise endogenously. This would perhaps be more confusing than helpful. Hence it has seemed best to conclude with Setchell and Gardner that it belongs in *Lophosiphonia.*

SUMMARY

1. *Hapalospongidion* should be recognized as a valid genus in the Ralfsiaceae.

2. *Pterochondria* is described as a new genus differing from *Pterosiphonia* in the flattened discoid antheridial branches similar to those of *Chondria* and in the lack of a creeping base.

3. *Taenioma clevelandii* Farlow is a valid species distinguished from *T. perpusillum* J. Ag. chiefly by the occurrence of the characteristic short flanking cells on all branches, including the prostrate rhizome, and by the rhizoids which are composed mostly of two cells rather than of one as in the type species.

4. *Gelidium caloglossoides* Howe occurs frequently along the coast of southern California.

5. *Lophosiphonia villum* (J. Ag.) S. & G. is somewhat intermediate between *Lophosiphonia* and *Polysiphonia.* Its inclusion in the first named genus seems to require a slight modification of Falkenberg's concept of the genus.

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BULLETIN OF THE TORREY BOTANICAL CLUB
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PANICUM BENNETTENSE, A NEW SPECIES FROM NORTH CAROLINA

WALTER V. BROWN

While botanizing near Durham, North Carolina, a panicum was collected which at first was taken for *P. angustifolium*. On closer examination, however, it was seen that it differed from that species in a number of particulars although belonging to the section Angustifolia. Professor H. L. Blomquist of the Department of Botany, Duke University, after a careful study of the material feels that it is an undescribed species. J. R. Swallen of the U.S.D.A. also expressed the view that it is a new species.

Panicum bennettense, W. V. Brown, sp. nov. (subgenus *Dicanthelium* Hitchc. & Chase, group *Angustifolia*). Planta caespitosa, 30–70 cm. alta; internodiis productis, 5–7, puberulis vel papillati-pubescentibus; nodis glabratis; vaginis sparse adscendenti-papillati-pubescentibus, superioribus tamen glabratis; statu vernali foliis 8–15 cm. longis, 4–8 mm. latis, planis, in longitudine sulcatis, productis acuminatisque, rigidis, ad basim paulo angustatis et ciliatis; foliis inferiorbus rigiduli-pubescentibus, superioribus scabris ad apicem involutam; ligulis ciliatis, 1–1.5 mm. longis; paniculis vernis longe exsertis, 5–9 cm. longis, 4–9 cm. diam.; rhachidibus glabratis; ramis late extensis, deinde postea adscendentibus; pedicellis productis, glabris; spiculis pubescentibus, 2 mm. longis, 1.2 mm. diam. ellipticis, apice acuminato, gluma inferiore 0.5 mm. longa, superiore et lemmate sterili aequilongis; fructu 1.7 mm. longo, 1.0 mm. diam.; statu autumnali ad basim rare ramoso sed ad nodos mediales atque superiores sparse ramoso, ramis adscendentibus, paniculis terminalibus, 1–2 cm. longis.

NORTH CAROLINA: Durham County, dry thickets in savannah at the Bennett Memorial, 5 miles west of Durham. TYPE in the Duke University Herbarium, *W. V. Brown 2492*.

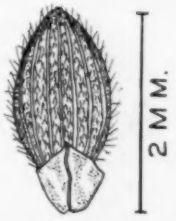

FIG. 1. Spikelet of *P. bennettense*

Plant 35–70 cm. high, erect, the lowermost internodes crisp papillose-pubescent, the middle and upper sparsely puberulent, the nodes glabrous; lower sheaths ascending-papillose-pubescent, the upper glabrous, ciliate; blades 8–15 cm. long, 4–8 mm. wide, stiff, flat, long acuminate, scarcely narrowed at the usually ciliate base, the lower often longitudinally wrinkled and shorter, harsh pubescent, the upper scabrous especially at the involute

tip; ligule 1–1.5 mm. long; panicle long exserted, 5–9 cm. long, 4–9 cm. in diameter, wide spreading at anthesis, later ascending, the axis glabrous; spikelets 2.0 mm. long, 1.2 mm. in diameter, ellipsoid, slightly attenuate at base, acuminate at tip, turgid; first glume about $\frac{1}{4}$ as long as the spikelet, glabrous, shining, pointed; second glume and sterile lemma equal, papillose-pubescent; fruit 1.7 mm. long, 1.0 mm. in diameter, elliptic. Autumnal culms stiffly ascending, sparsely branched at the middle and upper nodes, the branches ascending; blades numerous, flat, slightly involute at the long acuminate tip, appressed, thin; secondary panicles terminal, 1–2 cm. long, often reduced to 3 or 4 spikelets.

This species is very close to *P. angustifolium* Ell., differing mainly in spikelet characters. The spikelets of *P. angustifolium* are 2.5–2.8 mm. long, 1.4–1.6 mm. in diameter, obovate, blunt, the first glume $\frac{1}{3}$ the length of the spikelet. *P. bennettense* has spikelets 2.0 mm. long, 1.2 mm. in diameter, elliptic, pointed, the first glume $\frac{1}{4}$ the length of the spikelet. The ligule is longer and the branches of the inflorescence more ascending than in *P. angustifolium*.

Three stools of this species were collected on the dry, sandy, savannah-like park surrounding the Bennett Civil War Memorial. This park has a grass flora rich in species of *Panicum*, 30 or more mostly coastal-plain forms having been collected in the four or five acres. It is for this place, 5 miles west of Durham, that the species has been named.

Herbarium specimens were made of the three plants and given numbers *2492A*, *2517*, and *2472*. Half of the stool of number *2492A* was potted and grown to the autumnal phase. This was then made into herbarium specimens and numbered *2492B*. The description is based on these four collections. A portion of *2492A* in the vernal phase was chosen as the type, was so marked, and has been deposited in the Duke University Herbarium. Co-types are numbered *2492A* and *2492B*, consist of both vernal and autumnal phases, and are from the same plant as the type. Isotypes are numbered *2472* and *2517* and are specimens of plants in the vernal phase. Complete sets of these specimens have been deposited in the Duke University Herbarium, the U. S. National Herbarium, and the author's personal herbarium.

The writer wishes to thank Professor H. L. Blomquist and Dr. Jason R. Swallen for their opinions concerning the status of these plants; Professors F. A. Wolf and R. S. Rogers for help with the Latin diagnosis, Professor H. J. Oosting and Dr. Lewis E. Anderson for help in preparing the manuscript.

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THREE NEW CUSCUTAS FROM WESTERN NORTH AMERICA

T. G. YUNCKER

Cuscuta Runyonii Yuncker, sp. nov. Caules tenues aut modici. Flores 2–3 mm. longi a basi ad corollae sinum, glandulosi. Pedicellati aequantes aut longiores quam flores. Inflorescentiae umbellato-cymosae. Calycis lobi triangulares aut plus minus ovati, subacuti, appendiculati-corniculati. Corollae lobi ovato-lanceolati, acutissimi, tubo campanulato aut subgloboso aequilongi, reflexi, apices inflexi. Squamae fimbriatae ad stamina attingentes. Styli tenui, aequantes aut longiori quam ovarium depresso-globossum. Capsula depresso-globosa, non circumscissilis. Semina 4, ovala, circ. 1.5 mm. longa.

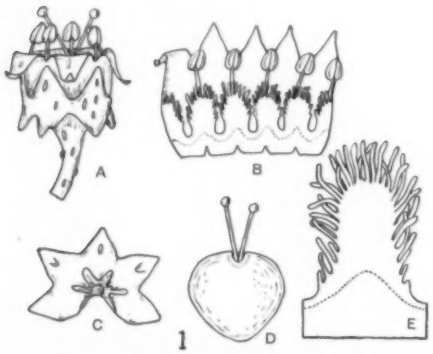
Stems slender to medium, smooth or often with scattered papillae near the inflorescence. Flowers "white" or "cream-white," drying yellow or reddish, 5-parted, mostly 2 to 3 mm. long from the base of the flower to the corolla sinuses, glandular, on pedicels varying in length, but commonly about equal to or somewhat longer than the flowers, in loose, umbellate cymes of 2 to 5 or 6 flowers, these ultimate clusters also sometimes umbellately arranged, bracts mostly 1 to 1.5 mm. long, oblong-ovate to triangular, acute, with a short, obtuse, saccate protuberance near the base. Calyx lobes triangular to somewhat ovate, obtuse or acutish, not overlapping at the base, about reaching the corolla sinuses, or shorter, each with a prominent, spur-like protuberance at the base and also frequently with a somewhat smaller one towards the apex. Corolla campanulate to subglobose, smooth or more or less scabrous, somewhat bulging between the lines of the filament attachments, lobes about as long as the tube, triangular-ovate to lanceolate, reflexed, acute to acuminate, tips inflexed, papillate. Stamens shorter than the corolla lobes, filaments slightly subulate and about equal to or slightly longer than the oval anthers. Infrastamineal scales prominent, reaching the stamens, profusely fimbriated, bridged below the middle. Styles slender, equal to or longer than the depressed-globose ovary, stigmas capitate. Capsules depressed-subglobose, smooth or papillate, not circumscissile, surrounded by the corolla. Seeds about 1.5 mm. long, oval, 4 in each of the capsules examined, embryo filiform, coiled.

TEXAS: Hidalgo County, occasional on dry clay hilltops, at La Joya, June 8, 1941, *Robert Runyon 2732*, TYPE, U. S. National Herbarium; occasional on dry clay hills, chaparral thickets, 2 miles north of La Joya, July 13, 1941, *Robert Runyon 2825*. (Very abundant material of the above two specimens permitted division and duplicates have been deposited in the Gray Herbarium, New York Botanical Garden, Field Museum of Natural History, and the Missouri Botanical Garden); Zapata County, 3¾ miles north of San Ygnacio, April 1, 1938, *V. L. Cory 28121* (Gray); Duval County, 21 miles northwest of San Diego, October 9, 1936, *V. L. Cory 17199* (Gray).

This species is remarkable because of the spurred calyx. The spurs, which have been observed on no other species, are sufficiently prominent to be readily visible with the unaided eye. They are mostly simple, sac-like protuberances tapering to an obtuse tip, but several were noted in which the

spurs were branched. They are nearly as wide at the base as the calyx lobe, or commonly narrower. Examination of hundreds of flowers failed to reveal any which were without spurs. Host plants include *Calophanes decumbens*, *Erigeron canadensis*, *Coldenia canescens*, and *Nama hispida*.

This species belongs in Subsection *Arvenses* of Section *Cleistogrammica* and appears to be most closely related to *C. glabrior* (Engelm.) Yuncker, a common species of the southwestern United States and northern Mexico. The prominently spurred and somewhat more triangular lobes of the calyx readily distinguish it from that species, however. From *C. appendiculata* Engelm. of South Africa it differs in its larger flowers, much larger projections on the calyx, character of the inflorescence, etc. It is named for Robert Runyon, collector of the type specimen.



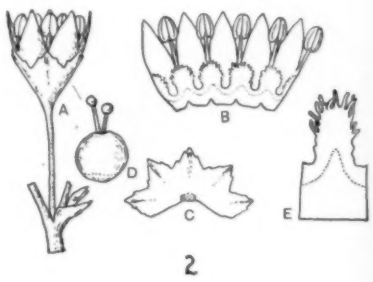


FIG. 1. *Cuscuta Runyonii* Yuncker, sp. nov. FIG. 2. *Cuscuta serruloba* Yuncker, sp. nov. *a*, flower × 10; *b*, opened corolla × 10; *c*, opened calyx × 10; *d*, fig. 1, capsule × 10; *d*, fig. 2, ovary × 10; *e*, individual scale × 20.

Cuscuta serruloba Yuncker, sp. nov. Caules tenuissimi. Flores 4- aut 5-divisi, circ. 1.5 mm. longi a basi ad corollae sinum. Pedicellati longiores quam flores. Calycis lobi triangulares, acuti, irregulares serrati. Corollae lobi oblongo-lanceolati, plus aut minus acuti. Scalae oblongae, fimbriatae, ad stamina attingentes. Filamenta plus aut minus subulati. Styli subulati breviores quam ovarium subglobosum. Capsula non visa, circumscissilis (?).

Stems very slender. Flowers 4- or 5-parted, about 1.5 mm. long from the base of the flower to the corolla sinuses, on pedicels mostly much longer than the flowers, in loose, mostly 3- to 5-flowered umbellate cymes, these in turn more or less paniculately arranged, bracts ovate, acute, denticulate. Calyx somewhat fleshy, lobes deltoid, not overlapping, subequal, medianally thickened and slightly crested near the acute apex, about reaching the corolla sinuses, edges irregularly toothed. Corolla lobes oblong-lanceolate, acutish, about as long as the campanulate tube, erect to spreading, margins entire or obscurely uneven. Stamens nearly as long as the corolla lobes, filaments stoutish, enlarging somewhat towards the base, commonly as long as or slightly longer than the comparatively large, oval-ovate anthers. Infrastamineal scales reaching the stamens, oblong, moderately fringed, bridged at about the middle. Styles stoutish and enlarging slightly toward the base, shorter than or about equal to the subglobose ovary, stigmas comparatively large, subglobose, smooth. No matured capsules are present on the material

examined but a line near the base of the ovary indicates that they are probably circumscissile.

MEXICO—COLIMA: Manzanillo, October 21, 1910, *C. R. Orcutt 4457*, TYPE, Gray Herbarium.

The material from which the above description is drawn is not abundant and does not contain any matured fruit. It appears evident, however, that this species belongs in Subsection *Umbellatae* of Section *Eugrammica*. It is apparently most closely related to *C. deltoidea* Yuncker, the type of which was collected in the same locality. It differs from that species, however, in the acute, non-overlapping and more triangular, serrulate calyx lobes, proportionately longer corolla lobes and the shorter (?) and stouter styles. From *C. lacerata* Yuncker it differs chiefly in the shape of calyx and corolla lobes and from *C. gracillima* Engelmann because of its shorter stamens and serrate calyx lobes.

CUSCUTA SALINA Engelmann var. **papillata** Yuncker, var. nov. Calycis et corollae lobi ovato-lanceolati, attenuati, acutissimi. Calycis et pedicellis superior pars papillosa.

Flowers about 3 mm. long from the base of the flower to the corolla sinuses. Calyx and corolla lobes ovate-lanceolate, attenuately acuminate. Calyx and upper part of the pedicel finely papillate. Anthers ovate-subsagittate, filaments subulate. Infrastamineal scales represented by narrow, sparingly fringed ridges up to about the middle of the corolla tube.

CALIFORNIA: Mendocino County, Fort Bragg, August 8–16, 1912, *Alice Eastwood 1593*, TYPE, Gray Herbarium.

This variety resembles the species in most respects. It differs principally in the papillate calyx and more attenuate perianth lobes.

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INDEX TO AMERICAN BOTANICAL LITERATURE

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

Papers that relate exclusively to bacteriology, forestry, agriculture, horticulture, manufactured products of vegetable origin, or laboratory methods are not included. If users of the Index will call the attention of the editor to errors or omissions, their kindness will be appreciated.

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PLANT TAXONOMY AND FLORISTICS
(exclusive of fungi)

(See also under Genetics: **Cave & Constance, Clausen, Epling & Dobzhansky, Göes**)

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